

**A CLINICAL STUDY ON RECURRENT APHTHOUS STOMATITIS:
AN ASSESSMENT OF MULTIPLE ETIOLOGICAL FACTORS**

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ABSTRACT: AIMS AND OBJECTIVES: To study the various etiological factors, the relation between haematinic deficiencies and recurrent aphthous stomatitis (RAS). **MATERIALS AND METHODS:** It was a prospective study to find out the aetiopathogenesis of RAS. A detailed history of 50 patients with RAS was taken with emphasis on etiological factors causing RAS such as age, stress, sleep, menstruation, trauma, constipation, smoking, drugs / food allergy and haematinic deficiencies. Diagnosis was based on history and clinical examination. Hemoglobin percentage, serum vitamin B12 & folic acid levels were seen for in blood. **RESULTS AND OBSERVATION:** We have evaluated 50 patients with RAS. Maximum numbers of patients were seen in the age group of 20 – 39 (68%). In our study 47 patients (94%) presented with minor aphthous ulcers and 3 patients (6%) with major ulcers. 64% of patients were from middle socioeconomic group, 70% of RAS was associated with anemia, 46% of stress related, 30% gave history of lack of sleep, 20% of female patients developed RAS during menstruation. Vitamin B12 deficiency (20%), trauma (22%), and 6% was associated with smoking, constipation and food allergy, 8% were found to be associated with alcohol. **CONCLUSION:** Recurrent Aphthous Stomatitis is a very common disease with several precipitating factors. Multiple combined etiological factors like lack of sleep, minor trauma to oral mucosa in susceptible individuals, menstruation, constipation, food allergies, vitamin B12 and folic acid deficiency etc. caused RAS. This data may be helpful in future studies regarding the aetiopathogenesis of RAS and to bring out better treatment or preventive options.

KEYWORDS: Recurrent Aphthous Stomatitis, Folic acid, Vitamin B12, Stress.

INTRODUCTION: Recurrent aphthous stomatitis (RAS) is the most common inflammatory ulcerative condition of the oral mucosa, which often occurs in otherwise healthy individuals. The term "aphthous stomatitis" has been used interchangeably with "aphthous ulcers" but at present, the term aphthous stomatitis is preferred.¹ Aphthous ulcers are painful, clearly defined, shallow, round or oval ulcers, with necrotic centers, covered with grey or yellow pseudomembrane and surrounded by raised margins and erythematous haloes.

Approximately 20% of general population is affected by RAS, but incidence varies from 5% to 50% depending on the ethnic and socioeconomic groups studied. It affects one in five of the world population at some time in their lives.² It carries no mortality but a high morbidity. RAS typically occurs in patients of age 10-30 years and recur at varying intervals throughout life. A higher prevalence has been found in the higher socio economic groups, in females, and among individuals with stress, such as students at the time of examinations and among professionals.²

Several factors have been proposed as possible causative agents of RAS. They include local factors, such as trauma in individuals who are genetically susceptible to RAS, microbial factors, and

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nutritional factors, such as deficiency of folate and B-complex vitamins, immunologic factors, psychosocial stress and allergy to dietary constituents.

Although it is one of the most common recurrent oral ulcerative conditions of adults and children recognized throughout the world, RAS is also one of the least understood oral diseases and is among the most vexing problem faced by affected patients and clinicians alike. Simple aphthosis is described when ulcer recurrences are few and not associated with systemic factors and occurs only 2–4 times each year.

Complex aphthosis is a disorder in which patients develop recurrent oral and genital aphthous ulcers or when there is a continuous disease activity with new lesions developing as older lesions heal, or when ulcers are associated with systemic diseases³. Major aphthae can be associated with human immunodeficiency virus (HIV) infection; clinicians should consider HIV testing when aphthae are large and slow to heal.⁴ In this scenario we conducted a prospective study to find out the aetio pathogenesis of aphthous stomatitis.

AIMS & OBJECTIVES: Our aim was to study the aetio pathogenesis of recurrent aphthous stomatitis, to find out the various aetiological factors causing this condition and to find out the relation between haematinic deficiencies and RAS.

MATERIALS AND METHODS: We conducted a prospective study in the Department of Otorhinolaryngology and Head-Neck Surgery, over a period of three years to find out the aetiopathogenesis of aphthous stomatitis.

Fifty patients with RAS were included in the study, with the following inclusion criteria like patients of any age and sex who had been suffering from aphthous stomatitis for at least 1 year with a frequency of at least 1 outbreak every 2 months. The following patients were excluded from the study - Patients who had known systemic diseases concurrent with lesions in the mouth (Behcet's disease, rheumatoid arthritis, lupus, and Acquired Immune Deficiency Syndrome), who had received treatment with vitamin B12 in any form for the last 1 year, who were pregnant or nursing and who had a known vitamin B12 deficiency.

A detailed history was taken from all patients with emphasis on aetiological factors causing aphthous ulcers; such as age, socio economic status, stress, sleep, menstruation, trauma, constipation, smoking, drugs/food allergy and haematinic deficiencies. A thorough examination of the oral cavity was done. Diagnosis was made based on history and clinical examination. The following blood tests were conducted haemoglobin, serum vitamin B12, serum folic acid and total cell count.

RESULTS & OBSERVATIONS: We have evaluated 50 patients with RAS. Maximum numbers of patients were seen in the age group of 20 – 39(68%). The youngest patient was of the age of 17, and the eldest was 66 years old. Males and females are equally affected by RAS. In our study 28% (14 cases) were in the high socioeconomic group, while 64% (32cases)and 8% (4cases)were seen in the mid and low socio-economic group respectively. Kuppuswami's classification was used to divide our patients into the three socio economic groups. Aphthous ulcers of size <1 cm is considered as minor and size >1cm is considered as major. 47 cases (94%) presented with minor aphthous ulcers and 3 cases (6%) with major aphthae. 46% of patients (27cases) gave a history of experiencing stress during the incidence of RAS. We used Pittsburgh Sleep Quality Index to quantify sleep. 30% (15cases)

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of RAS were seen to be precipitated by lack of sleep whereas 70% (35 cases) had normal sleep.

Out of the 25 females in our study 5 of them (20%) gave a history of development of RAS during menstruation. In 22% of cases the incidence of RAS were associated with minor trauma of the oral mucosa, such as tooth brush injury, self inflicted bites, sharp tooth and dental appliances.

Constipation was seen to be a precipitating factor in 6% of cases. Patients smoking more than one cigarette per day were considered as smokers in our study. 3 patients (6%) with RAS were smokers. Patients who had an alcohol consumption of >100 gms/week were considered as alcoholic. 8% of cases gave a history of alcohol consumption. But they denied any relation between alcohol use and incidence of RAS. 3 cases (6%) noticed that RAS occurred when they ingested pineapple and sea food. In our study we did not notice the development of RAS as a result of drug reaction.

Normal level of serum vitamin B12 is 220-1100 pg/ml. Levels <220pg/ml is considered as B12 deficiency and 220-500pg/ml is considered as low normal. RAS was seen in 37 patients (74%) with low normal and deficient vitamin B12 level compared to 13 patients with normal vitamin B12 level.

Normal level of serum folic acid is 3.1-20 ng/ml. Folic acid levels <3.1ng/ml is considered as folic acid deficiency and 3.1-5ng/ml is considered as low normal level. RAS was seen in 10 patients (20%) with low normal and deficient folic acid level compared to 40 patients with normal folic acid level.

Haemoglobin level <13 gm% in males and that of less than 12 gm% in females were considered as anaemic. In our study 35 patients (70%) were anaemic who developed RAS compared to 15 patients with normal haemoglobin levels.

The results of multiple etiological factors studied are given in the table - 1.

Aetiological factors		Present		Absent	
		Numbers	%	Numbers	%
Stress		23	46	27	54
Lack of sleep		15	30	35	70
Menstruation		5	20	20	80
Trauma		11	22	39	78
Constipation		3	6	47	94
Smoking		3	6	47	94
Alcohol		4	8	46	92
Food allergy		3	6	47	94
Anemia		35	70	15	30
Vitamin B - 12	Deficiency (<220pg/ml)	10	20		
	Low normal (220 - 500pg/ml)	27	54		
	Normal (220 - 1100pg/ml)	13	26		

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Folic acid	Deficiency (<3.1 ng/ml)	2	4
	Low normal ($3.1 - 5$ ng/ml)	8	16
	Normal ($3.1 - 20$ ng/ml)	40	80

Table 1: Aetiological factors of Recurrent Aphthous Stomatitis

DISCUSSION: Recurrent aphthous stomatitis (RAS) is the most common inflammatory ulcerative condition of the oral mucosa. Aphthous ulcers are painful, clearly defined, shallow, round or oval ulcers with necrotic centers covered with grey or yellow pseudo membrane and surrounded by raised margins and erythematous haloes. Aphthous ulcers of size <1 cm was considered as minor and size of >1 cm was considered as major. Forty seven (94%) patients presented with minor aphthous ulcers and only 3 patients 6(%) with major apthae. In a study conducted by McCullough et al ⁵ 87.1% had minor ulcers, 8.6% had major ulcers and 4.3% had herpetiform ulcers. The result of our study was similar to those studies done by other authors. We conducted a prospective study to find out the aetiopathogenesis of aphthous stomatitis.

In most patients the oral ulcers first appear in childhood or adolescence; 67-85% developing below the age 30 years. They typically recur with decreasing frequency and severity as the patient become older. Oral ulcers beginning or worsening well into adult life should increase the suspicion that the ulcers are being caused by an underlying medical disorder⁶. Waranu et al⁷ conducted a study on recurrent aphthous stomatitis in Tai dental patient's and found out that the incidence is maximum in 20-30 years population (34.9%). He also noticed that prevalence is slightly higher in women (48.2%) compared to men (44.1%). McCullough et al⁵ in his study noticed that males are affected more than females.

In our study also maximum incidence was found to be in 20-39 age groups (42% of cases) but there was no difference in incidence between males and females. Kuppaswami's classification was used to divide our patients into the 3 socio-economic groups. In our study we had 14 patients (28%) of higher class, 32 patients (64%) of middle class and 4 patients (8%) of lower socio-economic status. Patients of higher socio economic status, RAS is five times more prevalent and represents $>50\%$ of oral mucosal lesions.⁸ Crivelli et al⁸ in his study found out that RAS prevalence was significantly higher in children of high socio-economic status (19% versus 2%). This difference in prevalence have been ascribed to stress arising from greater expectations and/or more demanding responsibilities, but so far the aetiology is purely speculative.

Twenty seven patients (54%) were experiencing stress during the incidence of aphthous ulcers. Ship et al⁹ 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 disease. Further to support these findings, Miller MF¹⁰ in a 12 year retrospective investigation of 651 subjects with RAS noticed that the student years represent the highest levels of RAS. Pittsburgh Sleep Quality Index was used to quantify sleep. Fifteen patients (30%) had aphthous ulcers precipitated by lack of sleep this may be due to lack of sleep is associated with anxiety and stress. A study conducted by McCollough et al⁵ found a statistically significant relation with menstruation and RAS.

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In our study out of the 25 female cases 5 (20%) gave a history of developing RAS at the time of menstruation. A minority of patients relate their ulceration to the leuteal phase of the menstrual cycle. In a study by Edward et al¹¹ twenty percent of RAS were associated with menstruation, 26% remission occurred during third trimester of pregnancy with recurrence within one month postpartum. So there appears to be a subset of patients with RAS who consistently have the onset of their symptoms in the leuteal phase or in association with menstruation.¹² In our study 22% of cases had associated minor trauma to the oral mucosa, such as tooth brush injury, self inflicted bites, sharp tooth or dental appliances prior to the onset of RAS. David Wray et al¹³ conducted a study on the role of mucosal injury in initiating RAS. Buccal mucosa of 30 patients with RAS and 15 healthy controls were injured by a suture needle and with a tenaculum.

They were monitored for 7 days for development of ulcer. Altogether 26 lesions were induced in 13 patients whereas none occurred in the controls. Mechanically induced ulcers were clinically indistinguishable from those usually seen in patients except that they were smaller in size and healed more quickly. They confirmed that mechanically induced injury of oral mucosa may cause oral ulceration in people susceptible to aphthous stomatitis. We found only 3 cases (6%) smokers in our study group having aphthous ulcers. Binnur Tuzun et al¹⁴ conducted a study to find out the relation between RAS and smoking. Among 34 patients with RAS 8.8% were active smokers compared with a significantly higher percentage (25.2) among control subjects. Patients suffering from RAS usually are non-smokers and there is a lower prevalence and severity of RAS among heavy smokers as compared to moderate smokers.

Some patients reported onset of RAS after smoking cessation, while some reported control of RAS on reinitiating of smoking. The use of smokeless tobacco also is associated with significantly lower prevalence of RAS. Three cases (6%) noticed that aphthous ulcers occurred when they consumed pineapple or sea food. Some investigators have correlated the onset of ulcers to exposure to certain foods such as cow's milk, gluten, chocolate, nuts, cheese, azo dyes, flavorings agents and preservatives. In our study we did not notice the development of aphthous ulcer as a result of drug reaction. Nicorandil (potassium channel blocker), non steroidal antiinflammatory drugs especially piroxicam, cytotoxic drugs, alendronate, phenytoin, lamotrigine, mycophenolate, sirolimus, tiotropium, propranolol can produce oral ulcers, but mainly in the older age group.^{15,16}

In our study 10 cases (20%) showed vitamin B12 deficiency. In 27 cases (54%) RAS are associated with low normal B12 levels. Koybasi et al¹⁷ in their study found that 35.2% of patients had vitamin B12 deficiency. Field et al¹⁸ found that 21% of the children studied were having combined hematological (vitamin B12, folic acid, iron) deficiency. Piskin S et al¹⁹ evaluated serum iron, ferritin, folic acid and vitamin B12 levels in 35 patients with RAS and in 26 healthy controls. Vitamin B12 levels were significantly lower in subjects with RAS than in the controls. He concluded that vitamin B12 deficiency may be an etiological factor in RAS.

The relation between vitamin B12 and RAS is statically not significant ($p=0.276$). Even though the role of vitamin B12 in RAS remains unclear, the dramatic response to replacement therapy and higher incidence of RAS in cases of B12 deficiency suggest a direct role of this vitamin on the pathogenesis of RAS. The combined effect of vitamin B12 deficiency and stress precipitating RAS were seen in 33.3% of patients which is statistically and clinically significant ($P=0.038$).

In our study 2 patients (4%) had folic acid deficiency and 8 patients (16%) had folic acid in the low normal limit. Porter et al²⁰ in his study found that 5% of cases were having folic acid

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deficiency. Hutcheon et al ²¹in his study evaluated 328 patients with RAS and he found hematological deficiencies in 45 patients (13.7%), of which 15(4.57%) were deficient in folic acid. The relation between folic acid and RAS is statically not significant (p=0.174).

Iron deficiency anaemia is a major nutritional problem in India and many other developing countries. In our study 70% (35 cases) were anaemic. The relation between anaemia and RAS is statically not significant (p=0.063). Porter et al²² in their study showed a significantly low serum ferritin level (11.6%) in RAS patients compared with control group (4.9%). Wray et al ²³ reported iron deficiency to be very rare among patients with RAS. They also claimed that in RAS patients response to iron replacement therapy for iron deficiency took longer period than the combined iron, vitamin B12 and folic acid, which was attributed to the difficulty in reconstituting body stores of iron.

We also noted that the combined effect of iron deficiency and trauma to the oral mucosa causing RAS was significant (P value=0.044).

CONCLUSION: Recurrent Aphthous Stomatitis is a very common disease which carries no mortality but a high morbidity. The incidence varies from 5% to 50% depending on the ethnic and socioeconomic groups. RAS is a multifactorial disease and there are several precipitating factors of which stress is the most important. Other associated factors are lack of sleep, minor trauma to oral mucosa in susceptible individuals, menstruation, constipation and food allergies. Although the effect of vitamin B12 and folic acid deficiency was not significant; the combined effect of vitamin B12 deficiency with stress and that of iron deficiency with trauma precipitated RAS was seen to be statistically and clinically significant. This data may be helpful in future studies regarding the cause and pathogenesis of the disease and to sought out better treatment or preventive options.

REFERENCES:

1. Shulman JD. Prevalence of oral mucosal lesions in children and youths in the USA. *Int J Paediatr Dent.* 2005; 15: 89 –97.
2. Akintoye SO, Greenberg MS. Recurrent aphthous stomatitis. *Dent Clin North Am.* 2005; 49: 31–47.
3. Felice Femiano, MD, PhD, Alessandro Lanza, Guidelines for Diagnosis and Management of Aphthous Stomatitis. (*Pediatr Infect Dis J* 2007; 26: 728–732).
4. Shetty K. Thalidomide in the management of recurrent aphthous ulcerations in patients who are HIV-positive: a review and case reports. *Spec Care Dentist.* 2005; 25: 236 –241.
5. McCullough, Abdel-Hafeth, Crispian Scully; Recurrent Aphthous Stomatitis revisited; clinical features, associations, and new association with infant feeding practices. *J Oral Pathol Med* (2007) 36; 615-620.
6. J.S Rennie, P.C.Reade, C Scully; Recurrent Aphthous Stomatitis.; *British dental Journal* 1985; 159; 361.
7. Pongissawarnun W, Laohapand P: Epidemiologic study on recurrent aphthous stomatitis in a Tai dental population. *Community Dent Oral Epidemiol* 1991; 19; 52-53.
8. Crivelli MR, Aguas S; Influence of socio economic status on oral mucosa lesion prevalence in school children. *Community Dentistry Oral Epidemiology* 1988; 16; 58-60.
9. Irwin I, Ship; Socio economic status and recurrent aphthous ulcers; *JADA*; Vol 73; July 1966.

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10. Miller MF, Ship; A retrospective study of the prevalence and incidence of recurrent aphthous ulcers in a professional population; *Oral surgery Oral Medicine Oral Pathology*; 1977; 43; 532-537.
11. Edward A, Michel F, W. Boyd Lee; Recurrent Aphthous Stomatitis; *Journal of American Medical Association*; Vol 166: No.7:1966 May.
12. *Obstetrics Clinics of North America*; 1990; 17; 331-333.
13. David Wray; Role of mucosal injury in initiating recurrent aphthous stomatitis; *British Medical Journal*; vol 283; 1981; 1569-1570.
14. Binnur Tuzun, Roni wolf; Recurrent aphthous stomatitis and smoking; *International Journal of Dermatology*; 2000; 39; 358-360.
15. Scarlette, pascal Joly; Association of major aphthous ulcers and nicorandil; *The Lancet*; Vol 352; 1998.
16. Sigel, B Antonia; Medications can induce severe ulcerations; *JADA* Vol-122; 1991; 75-77.
17. Serap Koybasi, Parlak, Erdinc Serin; Recurrent Aphthous Stomatitis: investigation of possible etiologic factors; *American Journal of Otolaryngology - Head and Neck Medicine and Surgery*; 27; 2006; 229-232.
18. Anne Field, Emma Rotter; Clinical and hematological assessment of children with recurrent aphthous ulceration; *British Dental Journal*; 1987; 163; 19.
19. Piskin S, Sayan C, Durukan N, Senol M. *J Eur Acad Dermatol Venereol*. 2002 Jan; 16 (1): 66-7.
20. S. Porter, C Scully, S Flint; Recurrent Aphthous Stomatitis the Efficiency of Replacement Therapy In Patients With Underlying Hematinic Defficiencies; *Annals of Dentistry*; 1992; 51; 14-16.
21. A. W. Hutcheon, D. Ray, J. H. Dagg; Clinical and Haematological Screening in Recurrent Aphthae; *Postgraduate Medical Journal* (December 1978); 54; 779-783.
22. S Porter, C Scully, S Flint; Hematological status in recurrent aphthous stomatitis compared with other oral disease; *Oral surgery Oral Medicine Oral Pathology*; 1988; 66; 41-44.
23. D. Ray, M.M Ferguson; Recurrent Aphthae: Treatment with Vitamin B12, Folic acid, Iron; *British Medical Journal*, 1975; 2; 490-493.

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