

## Original Research Article

**A Hospital Based Prospective Study to Assess the Level of Vitamin B12 and Hemoglobin in Recurrent Aphthous Stomatitis Patients****Suchanda Das<sup>1</sup>, Ashok Kumar<sup>2</sup>, Umesh Chandolia<sup>3\*</sup>****<sup>1</sup>Senior Resident, Department of Dermatology, Venereology & Leprosy, S. K. Government Medical College & Attached Groups of Hospital, Sikar, Rajasthan, India****<sup>2</sup>Junior Specialist, Department of Dermatology, Venereology & Leprosy, S. K. Government Medical College & Attached Groups of Hospital, Sikar, Rajasthan, India****<sup>3</sup>Senior Resident, Department of Otorhinolaryngology, S. K. Government Medical College & Attached Groups of Hospital, Sikar, Rajasthan, India****Received: 09-07-2021 / Revised: 13-08-2021 / Accepted: 23-10-2021****Abstract**

**Background:** Recurrent aphthous stomatitis (RAS) is a chronic inflammatory disease of the oral cavity. The etiopathogenesis of RAS is not fully understood. Different etiologies and mechanisms have been postulated, resulting in identification of a variety of predisposing factors. In this study, we investigated the vitamin B12 and hemoglobin levels of patients with RAS and compared the results with healthy controls. **Materials & Methods:** This is a hospital based prospective study including 50 patients with RAS (RAS group) and 50 healthy controls (control group) in the Department of Otorhinolaryngology, and Department of Dermatology, Venereology & Leprosy at S.K. Government Medical College, Sikar, Rajasthan for a period of 8 months. Patients who had had at least one episode of oral ulcerations per month during the preceding years were diagnosed as having RAS. An equal number of healthy controls who were either attendant of patient or staff of hospital were enrolled in this study. Biochemical analysis was done before the RAS treatment was initiated. The results should be regarded as being significant if the  $P < 0.05$ . **Results:** In the RAS group, the mean age of patients was  $43.6 \pm 13.5$  years and  $42.50 \pm 14.23$  years in healthy controls. In our study, 15 (30%) patients were diagnosed as Hb deficient in the study group, as compared to 5 (10%) subjects in healthy controls ( $P < 0.05$ ). On the other hand, vitamin B12 deficiency was diagnosed in 16 (32%) subjects in the RAS group and 7 (14%) healthy controls ( $P < 0.05$ ). The RAS group had significantly lower mean Hb levels ( $P < 0.001$  for both men and women) than healthy controls. A significant difference in the mean vitamin B12 level was not demonstrated between the RAS group and the control group. **Conclusion:** We concluded that vitamin B12 and hemoglobin deficiencies were found higher in patients with RAS compared to the control group.

**Keywords:** Recurrent Aphthous Stomatitis (RAS), Ulcer, Vitamin B12, Hemoglobin.

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**Introduction**

Recurrent aphthous stomatitis (RAS), is a oral mucosal disorder, characterized by recurring ulcers with no other signs of disease, affecting one subject in five of the population at some time in their lives[1].

Recurrence of aphthous stomatitis is a chronic inflammatory disease of the oral cavity. It is one of the most common mucosal diseases of the oral cavity. Depending on the population, the increase varies from 5% to 20%[2].

RAS can be classified into 2 systems based on morphology, or clinical presentation. The first classification is based on morphology and through this category RAS can be categorized into three different categories according to their site, including Minor RAS, Major RAS, and Herpetiform RAS. About 80% of all RAS lesions are minor RAS[3].

The second classification of RAS is clinical, depending on the severity of the lesions. Simple aphthosis consists of few lesions that heal in one to two weeks and then recur[4]. Complicated aphthosis represents a complex clinical picture of a serious disease. Complex aphthosis produces ulcers continuously, with multiple lesions simultaneously lasting throughout the year. This type of RAS develops over time in life and is strongly associated with systemic diseases including behcet's disease, cyclic neutropenia, HIV AIDS, inflammatory bowel disease, and hematinic deficiency[4-6].

The etiopathogenesis of RAS is not fully understood. Investigators are still trying to determine the exact etiology of this traumatic event. The local and systemic conditions that can cause these lesions are widely investigated, but so far no major cause has been identified[5]. It has been suggested by the results of several studies that there is an important role of hematological parameters (hemoglobin, ferritin, hematocrit, folate and vitamin B12) in the pathogenesis of RAS, but there is still some controversy[7-11]. For better treatment planning it is important to recognize the lesions of aphthae secondary to systemic diseases like inflammatory bowel disease, pemphigus vulgaris, behcet's disease, and hematinic deficiencies[12]. In this study, we investigated vitamin B12 and hemoglobin levels in patients with RAS and compared the results with healthy controls.

**Materials & methods**

This is a hospital based prospective study comprising 50 patients with RAS (RAS group) and 50 healthy controls (control group) in the Department of Otorhinolaryngology and Department of Skin,

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#### Exclusion Criteria

1. RAS patients with concomitant Behçet's disease, coeliac disease, gluten-sensitive enteropathy, pemphigus.
2. Subjects with systemic disease or those taking any oral supplements.
3. Pregnant or lactating mothers.

#### Exclusion Criteria of Healthy Controls

1. Any oral mucosal or systemic disease.
2. History of intake of any medication like sulphonamide, rifampicin, vancomycin, NSAIDs or any cytotoxic drugs at least 3 months before the study.

#### Methods

Patients who had had at least one episode of oral ulcerations per month during the preceding years were diagnosed as having RAS[13]. Healthy controls were selected amongst attendants of patients & staff member of hospital. Biochemical analysis was done before the RAS treatment. Blood samples were drawn from the RAS group and control group for measuring blood Hb and vitamin B12 concentrations. With regard to the World Health Organization criteria, women with Hb <12 g/dl and men with Hb <13 g/dl were defined as

having Hb deficiency or anemia[14]. Moreover, patients with serum vitamin B12 level <200 pg/ml were defined as vitamin B12 deficient for both genders[15].

The blood Hb and vitamin B12 concentrations were investigated by an auto analyzer in the routine laboratory that was performed in the Department of Biochemistry Laboratory in S.K. Government Medical College, Sikar.

#### Statistical Analysis

Statistical analyses were performed using the SPSS software version 22. Continuous variables were expressed in mean  $\pm$  standard deviation, and nominal variables were shown in percentage. The Mann-Whitney U-test was used to compare the groups in respect of continuous variables while the difference between the groups was tested by Pearson Chi-square test in respect of nominal variables. The results should be regarded as being significant if the  $P < 0.05$ .

#### Results

In our study, majority of the patients with recurrent aphthous stomatitis (RAS) were in the age group between 21-40 years and females outnumbered males. (Table 1) There was no difference in terms of age between the RAS group and the control group. In the RAS group, the mean age of patients was  $43.6 \pm 13.5$  years and  $42.50 \pm 14.23$  years in healthy controls.

**Table 1: Age and sex distribution of patients with RAS**

Age (yrs)	Male	Female
0-20 yrs	1 (2%)	1 (2%)
21-40 yrs	7 (14%)	14 (28%)
41-60 yrs	5 (10%)	14 (28%)
>60 yrs	3 (6%)	5 (10%)
Total	16 (32%)	34 (68%)

In our study, 15 (30%) patients in the RAS group, and 5 subjects (10%) among healthy controls ( $P < 0.05$ ) were diagnosed as Hb deficient. On the other hand, vitamin B12 deficiency was diagnosed in 16 (32%) subjects in the RAS group and 7 (14%) healthy controls ( $P < 0.05$ ) (Table 2 & Figure 2,3).

**Table 2: Distribution of individual with Hb and vitamin B12 deficiency and normal level in study group and healthy controls**

Biochemical analysis	RAS group	Healthy control group	P-value
Hb. Deficiency (%)			
No	35	45	<0.05*
Yes	15	5	
Vitamin B12 deficiency (%)			
No	34	43	<0.05*
Yes	16	7	

The Hb and vitamin B12 levels were analyzed separately for men and women. Our results showed that the RAS group had significantly lower mean Hb ( $P < 0.001$  for both men and women) levels than healthy controls. On the other hand, a significant difference in the mean vitamin B12 level was not demonstrated between the RAS group and the control group (Table 3).

**Table 3: Mean blood concentrations of Hb and vitamin B12 in patients with RAS and in healthy controls**

Factors	RAS group	Healthy control group	P-value
<b>Hb. (g/dl)</b>			
Male	13.58 $\pm$ 1.26	14.73 $\pm$ 1.32	<0.001**
Female	11.24 $\pm$ 1.40	12.88 $\pm$ 1.35	<0.001**
<b>Vitamin B12 (pg/ml)</b>			
Male	258.27 $\pm$ 100.56	290.87 $\pm$ 128.5	>0.05
Female	295.44 $\pm$ 132.90	305.24 $\pm$ 106.3	>0.05

#### Discussion

Our results showed that RAS patients had significantly lower mean Hb levels ( $P < 0.001$ ) for both men and women than healthy controls. Moreover, 15 (30%) patients in the study group had Hb deficiency. On the other hand, 5 individuals (10%) among healthy controls had Hb deficiency. Anemia and lower Hb levels in RAS patients cause a reduction in the capacity of the blood to carry oxygen to the oral mucosa and eventually lead to atrophy of the oral mucosa[16]. Iron is

present in the structure of Hb and is essential for epithelial cell functions. Oral epithelial cells have a high turnover rate and folic acid and vit B12 play important roles in DNA synthesis and cell division[17]. Vitamin B12 deficiency affects especially hematological and neurological systems[18]. Therefore, vitamin B12, folic acid, and iron deficiencies can cause oral epithelial atrophy[16].

In our study, although mean vitamin B12 levels were not significantly lower in the RAS group than in the control group, vitamin B12

deficiency was significantly higher in the RAS group than in the control group ( $P < 0.05$ ). In the study group, 16 (32%) individuals had vitamin B12 deficiency and 7 (14%) individuals among healthy controls had vitamin B12 deficiency. In the literature, there has been a large number of studies about the prevalence of vitamin B12 deficiency in RAS patients [19]. In these studies, it has been stated that 0-42% of RAS patients may have vitamin B12 deficiency. Geographical and temporal variations in diet and food supplementation may affect this variation. Vitamin B12 acts as a coenzyme in fat and carbohydrate metabolism, hematopoiesis and protein synthesis [20]. It has been reported that vitamin B12 deficiency can suppress cell-mediated immunity and change the cells of the tongue and buccal mucosa [21].

Wray et al found low level of Serum B12 in 21.7% RAS patients as compared to 8.5% in controls [7]. The results of Wray were supported by a recent study carried out by Burgan SZ 2006, that showed 26.6% of RAS patients demonstrated vitamin B12 deficiency as compared to the control with B12 deficiency in 12.6% [22].

### Conclusion

Vitamin B12 and Hb deficiencies were found higher in patients with RAS compared to the control group. So, it is recommended that the RAS patients should be routinely screened for Hb or vitamin B12 deficiency for better treatment outcome.

### References

1. Besu I, Jankovic L, Konic-Ristic A. The role of specific cow's milk proteins aphthous ulcers. *J Oral Pathol Med* 2013; 42(1):82-8.
2. Lopez-Jornet P, Camacho-Alonso F, Martos N. Hematological study of patients with aphthous stomatitis. *Int J Dermatol* 2014;53:159-63.
3. Rogers RS III. Recurrent Aphthous Stomatitis: Clinical characteristics and associated systemic disorders. *Semin Cutan Med Surg* 1997; 16: 278-305.
4. Scully C, Gorsky M, Lozada-Nur F. The diagnosis and management of recurrent aphthous stomatitis A consensus approach. *JADA* 2003; 134: 200-207.
5. Scully C 2005. Aphthous ulcers. <http://www.emedicine.com/ent/topic700.htm> 3/6/2007.
6. Scully C, Bagan JV. Adverse drug reaction in orofacial region. *Crit Rev Oral Biol Med* 2004; 15: 221-239.
7. Wray D, Ferguson MM, Mason DK, Hutcheon AW, Dagg JH. Recurrent Aphthae: Treatment with Vitamin B-12, folic acid and Iron. *Br Med J* 1975; 2: 490-493.
8. Tyldesley WR. Stomatitis and recurrent oral ulceration: is a full blood screen necessary? *Br J Oral Surg* 1983; 21:27-30.
9. Barnadas MA, Remacha A, Condomines J, de Moragas JM. Hematologic deficiencies in patients with recurrent oral aphthae. *Med Clin* 1997; 109: 8587.
10. Oslan JA, Feinberg I, Silverman JR, Abrams D, Greenspan JS. Serum vitamin B12, folate and iron levels in recurrent aphthous ulceration. 1982
11. Palopoli J, Waxman J. Recurrent aphthous stomatitis and vitamin B-12 deficiency. *South Med J* 1990; 83: 475-477.
12. Scully C, Flint DH. Oral medicine-update for the dental Practitioners Aphthous and other ulcers. *BDJ* 2005; 199:259-264.
13. Sun A, Chang YF, Chia JS, Chiang CP. Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of recurrent aphthous ulcerations. *J Oral Pathol Med* 2004;33:133-9.
14. WHO/UNICEF/UNU. Iron deficiency anaemia assessment, prevention, and control: A guide for programme managers. Geneva, Switzerland: World Health Organization; 2001.
15. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and Vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr* 2007;85:193-200.
16. Sun A, Chen HM, Cheng SJ, Wang YP, Chang JY, Wu YC, *et al*. Significant association of deficiencies of hemoglobin, iron, Vitamin B12, and folic acid and high homocysteine level with recurrent aphthous stomatitis. *J Oral Pathol Med* 2015;44:300-5.
17. Lahner E, Annibale B. Pernicious anemia: New insights from a gastroenterological point of view. *World J Gastroenterol* 2009;15:5121-8.
18. Incecik F, Herguner MO, Altunbassak S. Deficiency of Vitamin B12 in Children and Epilepsy. *Balkan Med J* 2010;27:351-3.
19. Baccaglini L, Lalla RV, Bruce AJ, Sartori-Valinotti JC, Latortue MC, Carrozzo M, *et al*. Urban legends: Recurrent aphthous stomatitis. *Oral Dis* 2011;17:755-70.
20. Kozlak ST, Walsh SJ, Lalla RV. Reduced dietary intake of Vitamin B12 and folate in patients with recurrent aphthous stomatitis. *J Oral Pathol Med* 2010;39:420-3.
21. Volkov I, Rudoy I, Abu-Rabia U, Masalha T, Masalha R. Case report: Recurrent aphthous stomatitis responds to Vitamin B12 treatment. *Can Fam Physician* 2005;51:844-5.
22. Burgan SZ, Sawair FA, Amarin ZO. Hematological status in patients with recurrent aphthous stomatitis in Jordan. *Saudi Med J* 2006; 27(3): 381-384.

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