



Clinical Profile of Recurrent Aphthous Ulcers and its Association with Serum Vitamin D and Vitamin B12 levels. A Cross-Sectional Study in Tertiary Care Hospital, Mandya

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Abstract

Introduction: Aphthous ulcers are recurrent oral immunological ulcers that are multifactorial in origin and arise as a result of T-cell mediated damage to the mucosal epithelial basal layer. Recurrent aphthous stomatitis (RAS) based on the clinical features, divided into three main types: minor aphthae (MiRAS), major aphthae (MaRAS) and herpetiform aphthae (HpRAS). Deficiencies of vitamin B12, folate, iron, ferritin, Vitamin D, vitamin E, vitamin B1, vitamin B2, and vitamin B6 have been reported in a portion of RAS patients.

Methodology: The study is a Cross-Sectional Study conducted in Department of Otorhinolaryngology. The study had 45 patients who were diagnosed with recurrent aphthous ulcer. All the data were collected in a specially designed case proforma. The diagnosis was made on the basis of a characteristic RAS clinical picture during an examination and the history of a regular mode of recurrence of the lesions.

Results: The study consisted of 45 patients of aged between 19 years to 57 years, of which 24 were males and 21 were females. The most common symptom the patient presented was burning sensation 62%. Out of 45 patients, 19 were found deficient, 9 patients had borderline serum levels for serum vitamin B12 levels and 10 patients had deficiency, 22 patients had insufficient levels for serum vitamin D levels.

Conclusion: This research provides a significant contribution to the literature regarding levels of vitamin D and vitamin B12 in patients with recurrent aphthous stomatitis. Screening aphthous ulcer patients by measuring serum vitamin D and vitamin B12 levels is necessary, as to prevent recurrent aphthous ulcer.

Keywords: Aphthous ulcers, Recurrent aphthous stomatitis, minor aphthae, major aphthae, herpetiform aphthae, Vitamin B12, Vitamin D.

INTRODUCTION

Aphthous ulcers are recurrent oral immunological ulcers that are multifactorial in origin and arise as a result of T-cell mediated damage to the mucosal epithelial basal layer. The most characteristic symptom of the disease is the recurrent onset of single or multiple painful erosions and ulcers that appear mainly on unattached oral mucosa of the lips, cheeks and tongue.^[1] The aphthous ulcers are surrounded by a characteristic erythematous halo and covered with fibrous coating. Recurrent aphthous stomatitis (RAS) based on the clinical features, divided into three main types: minor aphthae (MiRAS), major aphthae (MaRAS) and herpetiform aphthae (HpRAS).^[2] Risk factors of recurrent aphthous stomatitis are local trauma, emotional or physiologic stress, toxin exposure, vitamin deficiency, allergy, poor oral hygiene, menstruation, and alterations in the oral flora.^[3] Although several etiological factors have been proposed, the exact cause underlying RAS remains unclear. In addition to immune dysregulation, multiple nutritional deficiencies, separately or in combination, are also reported to be the possible etiologies of RAS. Deficiencies of vitamin B12, folate, iron, ferritin, Vitamin D, vitamin E, vitamin B1, vitamin B2, and vitamin B6 have been reported in a portion of RAS patients, deficiencies may be involved in keratinocyte proliferation and immune imbalance, thereby inducing RAS.^[4] Vitamin B12

deficiency suppresses the cell mediated immunity and is the most frequently found haematinic deficiency, producing changes in the epithelium of the oral cavity. Serum vitamin B12 is essential for the synthesis of DNA, and its deficiency results in the development of megaloblastic anaemia, especially in developing countries including India. ^[5,6] Vitamin D (VD) is a fat-soluble secosteroid and functions primarily in the regulation of the calcium and phosphorus balance. Growing body of evidence suggests that VD and vitamin D receptor (VDR) are directly involved in T-cell antigen receptor signaling, indicating their role in the anti-inflammation processes. Therefore, defects in VD/VDR may be associated with the body's normal response to bacterial infections and inflammations. An association between VD level and idiopathic oral mucosal disorders, such as RAS, has the potential of improving our understanding of the immunological steps that lead to the development of aphthous ulcers. ^[7-11]

METHODOLOGY

The study is a Cross-Sectional Study conducted in Outpatient Department, Department of Otorhinolaryngology, Mandya institute of medical sciences, Mandya, during the period of 2 Months (from October 2023 to December 2023). The study had 45 patients who were diagnosed with recurrent aphthous ulcer. All the data were collected in a specially designed case proforma. Informed consent was taken from all the patients. All patients complained of burning sensation, pain in oral cavity, difficulty in swallowing were included in the study. The diagnosis was made on the basis of a characteristic RAS clinical picture during an examination and the history of a regular mode of recurrence of the lesions. RAS was further divided into major-typed RAS, minor-typed RAS and Herpetiform RAS. ^[1]

Type of aphthous ulceration	Clinical features
Minor aphthous ulceration	<ul style="list-style-type: none"> ● Most common form of aphthous ulceration ● Less than 10 mm in diameter ● Last 2–3 weeks ● Only on non-keratinized mucosa ● Heal without scarring ● Can be multiple but fewer than 10 at a time ● Usually at the front of the mouth ● Commonly in areas of mucosal trauma
Major aphthous ulceration	<ul style="list-style-type: none"> ● Larger than 10 mm in diameter ● Can last up to 3 months ● Can occur on keratinized or non keratinized mucosa ● Can heal with or without scarring ● Usually single but 1–3 ulcers can be present at once ● Usually towards the back of the mouth
Herpetiform ulceration	<ul style="list-style-type: none"> ● Least common form of aphthous ulceration ● Less than 5 mm in diameter ● Last for up to 2 weeks ● Heal without scarring ● Large numbers of ulcers – up to 100 ● Coalesce in areas to form large areas of ulceration ● Arise in keratinized and non-keratinized mucosa ● Similar in appearance to primary herpetic gingivostomatitis

Table No 1: – Types of Ulcers and Clinical Features

Inclusion Criteria:

- Patients with recurrent oral ulceration aged between 18 -60 years attending Department of otorhinolaryngology, MIMS Mandya.
- Patients willing to give informed consent to participate in the study.

Exclusion Criteria:

- Patients who are known chronic smokers and alcoholics.
- Patients with history of medical disorders such as hypertension, hepatic, renal, cardiac disorders, Behcet's disease, 45 aematological disorders, Crohn's disease and ulcerative colitis, systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, pemphigus vulgaris, and cicatricial pemphigoid.
- Patients on medications such as sulphonamides, rifampicin, vancomycin, cytotoxic agents like methotrexate and non-steroidal anti-inflammatory drugs.
- Patients with mental disorders, cognitive impairments.

The blood samples were drawn from RAS patients for the measurement of blood vitamin B12 and vitamin D levels. A serum vitamin B12 above 300 pg/mL is normal, between 200 and 300 pg/mL are considered borderline and below 200 pg/mL are considered deficient.^[13] A vitamin D level of 30-100ng/ml is evaluated as sufficient, 10 to 30 ng/ml as insufficient, and below 10 ng/ml as a deficiency.

Statistical analysis:

All collected data were entered in excel sheet and the data was statistically analysed by using SPSS software trail version 22. Descriptive statistical tests like frequency, proportion, for categorical data, mean, standard deviation for quantitative data like age, serum vitamin B12 levels, serum vitamin D level and inferential statistical tests like chi square test, Spearman's rank correlation to know the association of RAS with levels of vitamin B12 and vitamin D. Significance level was considered at 5 % ($p < 0.05$).

RESULTS

The study consisted of 45 patients of aged between 19 years to 57 years, of which 24 were males and 21 were females.

Symptoms: (Fig I)

Symptoms	Males (24)	Females (21)	Total (45)
Burning sensation	14 (58%)	14 (67%)	28 (62%)
Throat irritation	9 (37%)	8 (38%)	16 (35%)
Difficulty in swallowing	10 (41%)	6 (28%)	16 (35%)
Pain	10 (41%)	11 (52%)	21 (47%)

Table No 2: - Symptoms

The most common symptom the patient presented was burning sensation 62% followed by pain 47%, difficulty in swallowing and throat irritation 35%. (Table 2)

The most common clinical presentation of ulcer was the minor form 82% followed by major form 11% and herpetiform ulcer 7% (fig no III-V). (Table 3)

Type of RAS	Males (24)	Females (21)	Total (45)
Minor RAS	18 (75%)	19 (90%)	37 (82%)
Major RAS	4 (17%)	1 (5%)	5 (11%)
Herpetiform RAS	2 (8%)	1 (5%)	3 (7%)

Table No 3: - Types of Ulcers and Presentation

Site of Involvement: (Fig II) (Table 4)

Site of Involvement	Males	Females	Total
Lip	8	6	14
Gums	7	4	11
Anterior 2/3 of tongue	12	8	20
Buccal mucosa	9	5	14
Hard palate	3	3	6
Gingivolabial sulci	5	4	9
Gingivobuccal sulci	3	3	6
Retromolar Trigone	3	3	6
Floor of mouth	4	2	6
Anterior pillars	2	2	4
Posterior pillars	2	1	3
Soft palate	7	3	10
Uvula	1	1	2
Posterior pharyngeal wall	3	2	5

Table No 4: – Site of Involvement

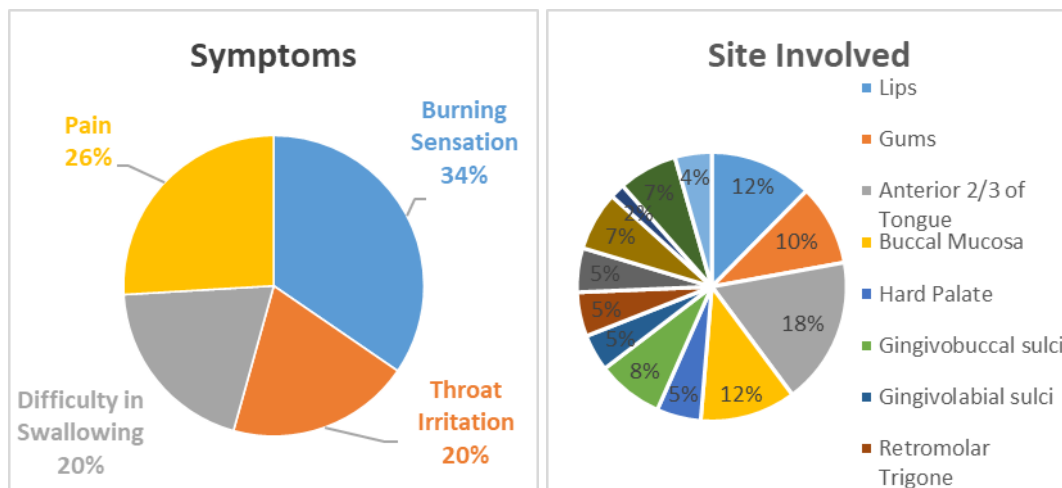


Fig I

Fig II

The most common site involved was tongue 18% followed lips 12%, buccal mucosa 12%, gums 10% and follows. Frequency of recurrence: The mean number of episodes was 4.48 ± 0.65 .

Severity of lesion and sites of involvement:

- Minor RAS - commonly involved sites are anterior 2/3rd of tongue, lips, gums and buccal mucosa.
- Major RAS- commonly involved sites are anterior 2/3rd of tongue and buccal mucosa.
- Herpetiform RAS- commonly involved sites palate and tongue.

Serum Vitamin B12 Levels: (Fig VI, VII) (Table 5)

Out of 45 patients, 19 were found deficient, 9 patients had borderline serum levels. The mean serum vitamin B12 level was 290.75 ± 44 pg/ml, with maximum level was 1076 pg/ml and minimum level was 90 pg/ml.

Type of RAS	Serum vit B12 levels in Males	Serum vit B12 levels in Females
Minor RAS	299.58±54.09	300.76±54.09
Major RAS	289.67±54.09	200
Herpetiform RAS	312.56	194

Table No 5: - Types of RAS with Serum Vitamin B12 levels



Fig III: Minor RAS



Fig IV: Major RAS



Fig V: Herpetiform RAS

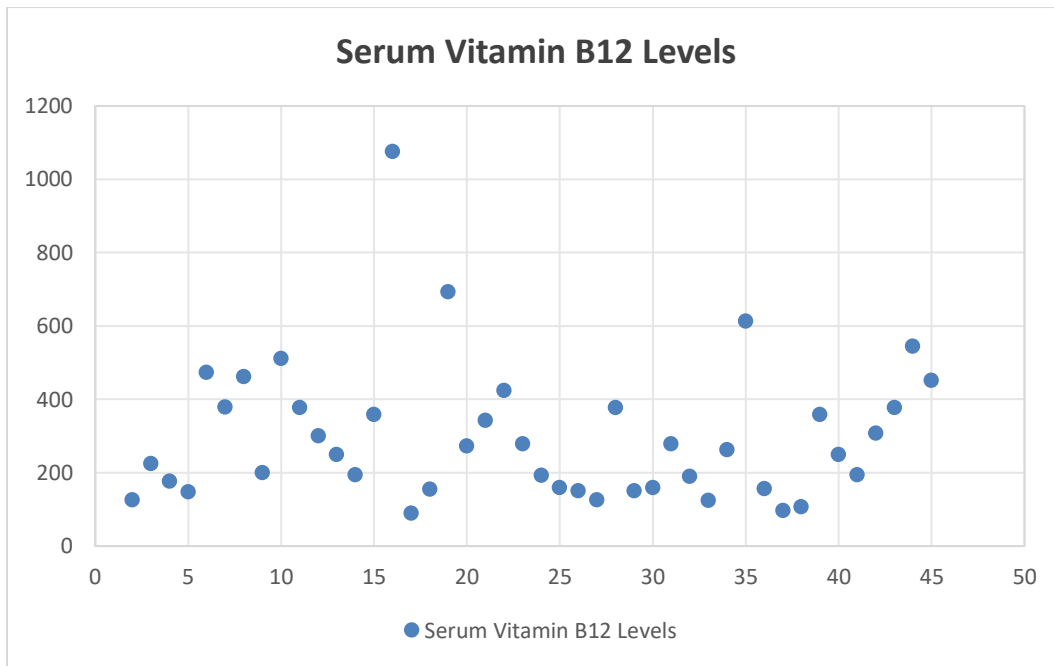


Figure VI: – Showing the levels of Vitamin B12 levels in Patients with RAS

Serum Vitamin D levels: (fig no VII) (Table 6)

A vitamin D level of 30-100ng/ml is evaluated as sufficient, 10 to 30 ng/ml as insufficient, and below 10 ng/ml as a deficiency. Out of 45 patients, 10 patients had deficiency, 22 patients had insufficient levels. The mean serum vitamin D level was 17.36 ± 2.53 ng/ml.

Type of RAS	Serum vit D levels in Males	Serum vit D levels in Females
Minor RAS	19.61 ± 5.47	23.49 ± 5.47
Major RAS	17.61 ± 5.47	12.2
Herpetiform RAS	12.02 ± 5.47	20.5

Table No 6: – Types of RAS with Serum Vitamin D levels

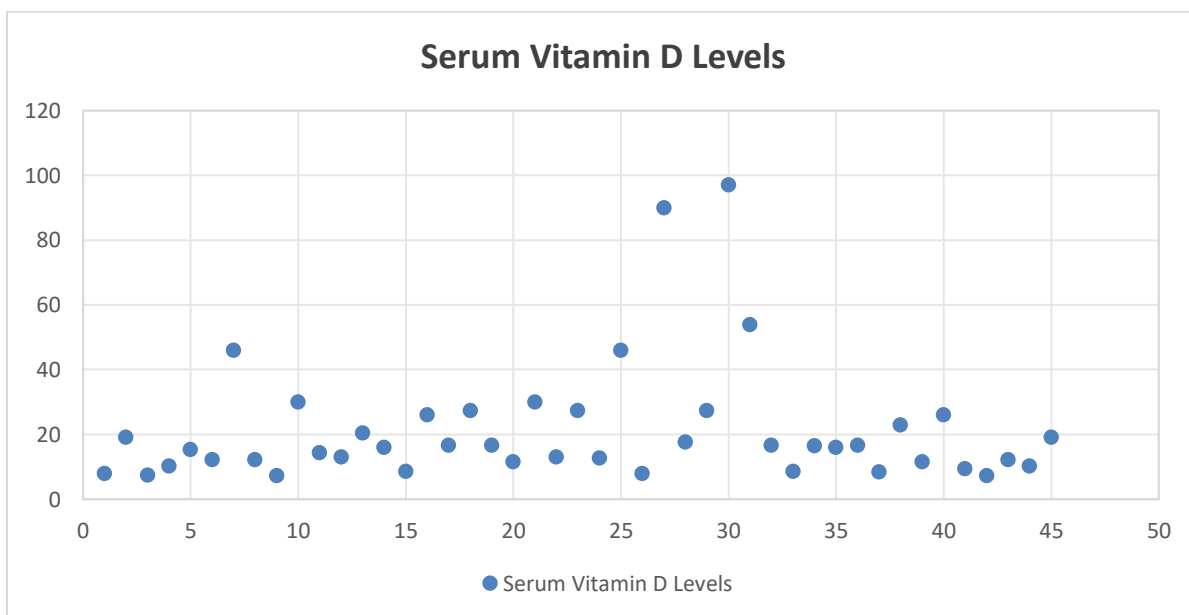


Fig VII: - Showing serum vitamin D levels in Patients with RAS

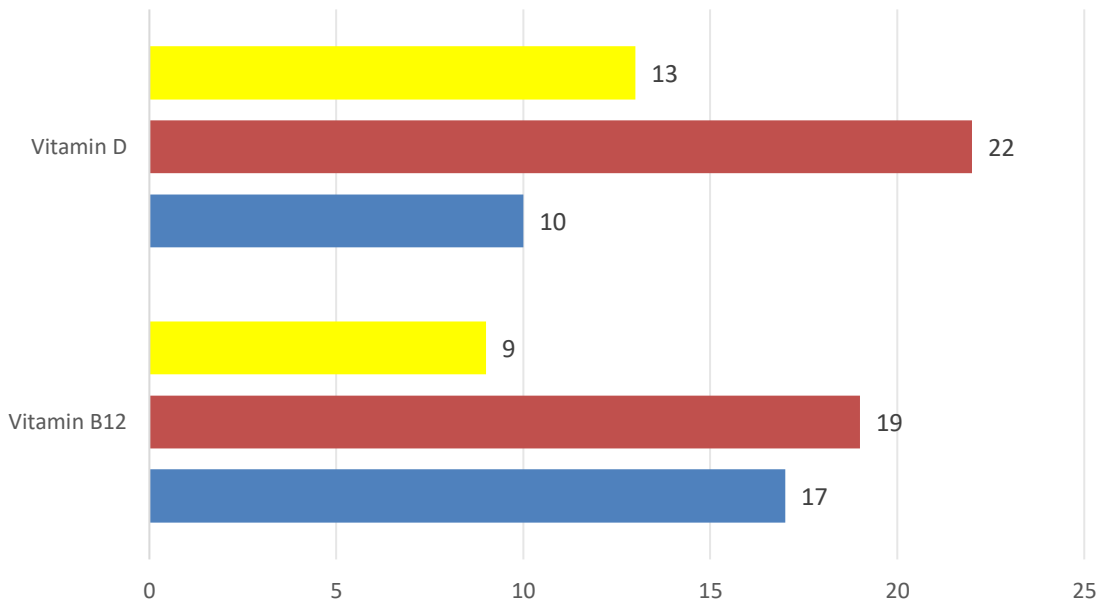


Fig VIII - Graph representing patients with normal serum levels (blue), Borderline levels(yellow) and deficient serum levels (brown).

DISCUSSION

A study conducted by Ślebioda Z et.al., across Poland during 2010-13 showed crucial role of the immunologic disturbances and genetic predisposition in the etiopathogenesis of RAS. ^[2]

The various studies conducted by Öztekin A et.al., Sun A et.al., Tidgundi MS et.al., across Turkey, Taiwan, Kalburgi Karnataka during the period of 2014-18 show various risk factors associated with recurrent aphthous ulcer were local trauma, emotional or physiologic stress, allergy, toxin exposure, vitamin deficiency, poor oral hygiene, menstruation, and alterations in the oral flora. ^[3-5]

The various studies conducted by Volkov I et.al., Al-Amad SH et.al., Krawiecka E et.al across Israel, United Arab Emirates, Poland during the period of 2005-17 shows nutritional deficiencies of vitamin B12, folate, iron, ferritin, Vitamin D, vitamin E, vitamin B1, vitamin B2, and vitamin B6 have been reported in a portion of RAS patients. ^[6-8] In our study serum vitamin B12 levels of 45 patients, 19 were found deficient, 9 patients had borderline serum levels. The mean serum vitamin B12 level was 290.75 ± 44 pg/ml.

The various studies conducted by Ślebioda Z et.al., Hussein FF et.al., Başarslan F et.al., across Poland, Turkey, Iran, Saudi Arabia, U.A.E during 2016-22 show vitamin D and vitamin D receptor are directly involved in T-cell antigen receptor signaling, indicating their role in the anti-inflammation processes. Therefore, defects in VD/VDR may be associated with the body's normal response to bacterial infections and inflammations. ^[9-11] Vitamin D levels were significantly low in children with recurrent aphthous stomatitis, suggest that low vitamin D levels may be associated with recurrent aphthous stomatitis. ^[11] In our study serum vitamin D levels of 45 patients, 10 patients had deficiency, 22 patients had insufficient levels. The mean serum vitamin D level was 17.36 ± 2.53 ng/ml.

A study conducted by Queiroz SI et.al., across Brazil during 2003-14 shows, RAS is divided into three forms: minor, major and herpetiform. The most common clinical presentation, the minor form > major form > herpetiform ulcer. The main reported symptoms by patients were pain (50%) followed by burning pain (25%) and other symptoms such as dysphagia, dysgeusia, and stinging pain (13.2%). And, in 11.7% of the cases, no symptoms were reported. Tongue being most common site affected followed buccal mucosa. ^[12] In our study most common symptom the patient presented was burning sensation followed by pain, difficulty in swallowing and throat irritation. The most common clinical presentation of ulcer was the minor form followed by major form and herpetiform ulcer. The most common site involved was tongue 18% followed lips 12%, buccal mucosa 12%, gums 10% and follows.

CONCLUSION

This research provides a significant contribution to the literature regarding levels of vitamin D and vitamin B12 in patients with recurrent aphthous stomatitis. In the present study, 62.2% of the patients had low serum vitamin B12 levels and 71 % of the patients had low and insufficient serum vitamin D levels. Screening aphthous ulcer patients by measuring serum vitamin D and vitamin B12 levels is necessary, as to prevent recurrent aphthous ulcer. It is also essential for recurrent aphthous ulcer patients to have nutritious diet containing vitamin D and vitamin B12. The long-term strategy for controlling B12 deficiency is to promote consumption of foods rich in Vitamin B12, mainly animal products, milk and dairy products and fish.^[14] The main reason for Vitamin D deficiency is inadequate sun exposure and insufficient consumption of vitamin D. Patients with fat malabsorption have higher rate of vitamin D deficiency since the vitamin D absorption is incomplete in this patients. About 10 to 15 minutes of sun on the arms and legs a few times a week can generate nearly all the vitamin D and the food sources such as milk or cheese should be fortified with vitamin D.^[14]

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