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A Horizontal Analysis of Vitamin A, D, E and B12 Levels in Serum among Recurrent Aphthous Stomatitis Patients and Normal Individual

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ABSTRACT
To analyze vitamin A, D, E and B12 levels in serum among recurrent aphthous stomatitis patients and normal individual. In patients with RAS and normal subjects, vitamin A, D, E and B12 levels was compared. The most common site of occurrence of aphthous ulcers was labial mucosa in 26, buccal mucosa in 14, tongue in 10 and gingiva in 8 cases. The difference was significant ($p<0.05$). The most common type of RAS was aphthous minor in 40, major in 11 and herpetiform in 7 cases The difference was significant ($p<0.05$). The mean vitamin A level was 14.3 mcg dL^{-1} in group 1 and 25.8 mcg dL^{-1} in group 2. The mean vitamin D level was 17.8 ng L^{-1} in group 1 and 26.2 ng L^{-1} in group 2. The mean vitamin E level was 8.2 µg mL^{-1} in group 1 and 11.5 µg mL^{-1} in group 2 and B12 was 102.4 pg mL^{-1} in group 1 and 436.8 pg mL^{-1} in group 2. The difference was significant ($p<0.05$). No significant correlation was found among the serum level of vitamin except between vitamin B12 and vitamin E in group 1. Vitamin A, B12, D3 and E deficiencies have a substantial impact on RAS and there is a significant link between RAS patients' serum levels of B12 and E.

INTRODUCTION

An idiopathic, acute, non-traumatic, inflammatory illness known as RAS is described as having painful, recurring ulcerative lesions without vesiculation as its precursor[1]. The moveable oral mucosa is damaged by RAS, which manifests as well-defined, round or oval, shallow/moderately deep ulcers of varied sizes, either as a single lesion or several lesions in a cluster, with the surrounding areas remaining clinically unaffected. The lesions consist of a raised halo of erythema at the periphery and a central necrosis region covered by a yellow-greyish pseudo-membrane. Major, minor and herpetiform aphthous are the classifications for RAS[2].

The precise pathogenesis of RAS is still unknown, studies indicate a multifactorial origin. Recent studies show that disruptions in the control of the immune-inflammatory response of the oral mucosa are crucial. Again, vitamins are crucial for immunological processes because they act as catalysts in several metabolic processes[3]. The immune-competent cells involved in innate/non-specific immunity, including polymorph, macrophages and NK cells, are all maintained in function by vitamin A. It also protects the integrity of the oral epithelium, which acts as the body's principal defense against external assaults. Additionally, vitamin A enhances the activities of B cells and CD4+cells, supporting antibody-mediated adaptive immunity. In order to maintain the CD4/CD8 ratio and NK cells in cellular immunity, vitamin B12 functions as an immune-modulator. Vitamin D3 regulates T-cell mediated cytotoxicity and CD8+cell proliferation[4].

Vitamin D3 regulates T-cell mediated cytotoxicity and CD8+cell proliferation. Vitamin E inhibits VLA4's ability to bind to the endothelium by reducing the release of reactive oxygen species by macrophages and the expression of CD11b cells and VLA4[5]. Additionally, vitamin E inhibits macrophage production of pro-inflammatory cytokines such as IL-1, IL-6 and IL-8TNF[6]. We performed this study to analyze vitamin A, D, E and B12 levels in serum among recurrent aphthous stomatitis patients and normal individual.

MATERIALS AND METHODS

After considering the utility of the study and obtaining approval from ethical review committee, we selected fifty- eight cases of recurrent aphthous stomatitis of both genders. All gave history of ulceration at least 3 times in last 3 months. Patients' consent was obtained before starting the study.

Data such as name, age, gender etc. was recorded. RAS patients were put in group 1 and healthy age and sex-matched subjects in group 2. Following a thorough clinical examination and the determination of the diagnosis of recurrent aphthous, 4 ml of venous blood was aspirated from the antecubital fossa using a 23 G needle and a 5 ml syringe, collected in clot and fluoride vials, labeled and stored in an ice box. The regular people underwent the same process once more. After coagulating and removing the blood clot from the samples, serum was extracted from the clot by spinning it in a centrifuge for five minutes at a speed of 3000 rpm.

For the 25-hydroxyvitamin D assay, serum samples were transferred to aliquot vials and stored at -20°C. All the reagents were brought to room temperature for at least 30 min before use for the initial estimation of vitamins A, B12, D3 and E. Following the manufacturer's instructions, the solid-phase sandwich ELISA kit was used to measure the serum vitamin A concentration. The methods used to estimate vitamin B12, vitamin D3 and vitamin E were remarkably similar to the method used to estimate vitamin B12. The results were compiled and subjected for statistical analysis using Mann- Whitney U test. P value less than 0.05 was set significant.

RESULTS

The most common site of occurrence of aphthous ulcers was labial mucosa in 26, buccal mucosa in 14, tongue in 10 and gingiva in 8 cases. The difference was significant ($p<0.05$) (Table 1 and Fig. 1).

Table 1: Distribution based on site

Site	No.	p-value
Buccal mucosa	14	0.05
Gingiva	8	
Tongue	10	
Labial mucosa	26	

Table 2: Distribution based on type

Type	No.	p-value
Major	11	0.02
Minor	40	
herpetiform	7	

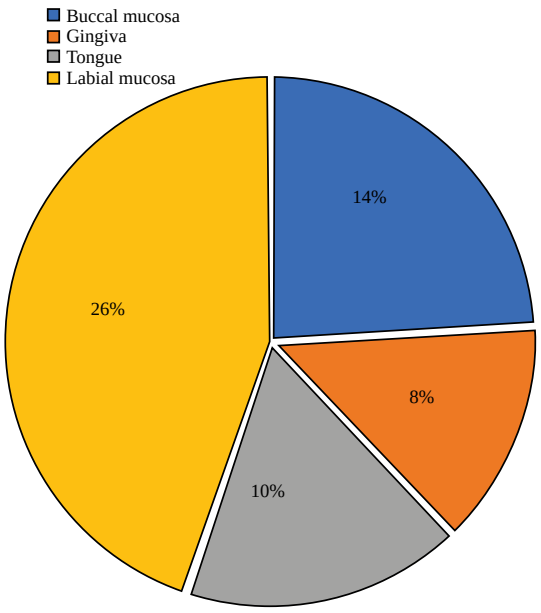

Fig. 1: Distribution based on site

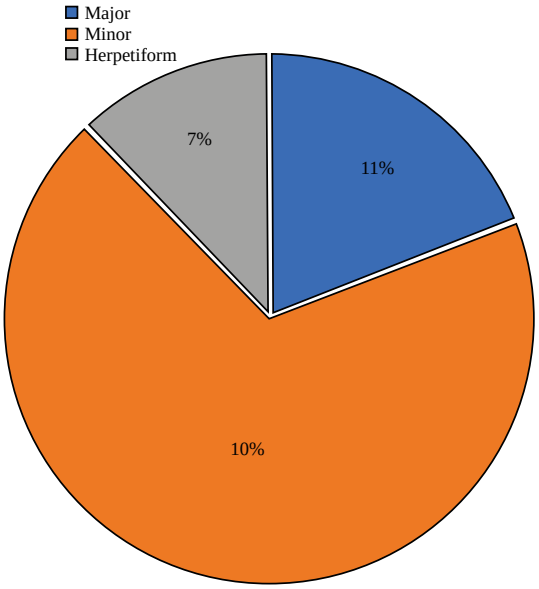

Fig. 2: Distribution based on type

Table 3: Estimation of vitamin A, D, E, B12

Vitamins	Group 1	Group 2	p-value
A (mcg dL^{-1})	14.3	25.8	0.010
D (ng L^{-1})	17.8	26.2	0.040
E (µg mL^{-1})	8.2	11.5	0.020
B12 (pg mL^{-1})	102.4	436.8	0.001

Table 4: Correlation between vitamin B12 and vitamin A/vitamin D3/vitamin E

	Group 1 (vitamin B12)		Group 2 (vitamin B12)	
Vitamins	r	p	r	p
A	-0.14	0.15	-0.19	0.84
D	-0.21	0.27	0.22	0.72
E	0.30	0.02	0.10	0.38

The most common type of RAS was aphthous minor in 40, major in 11 and herpetiform in 7 cases The difference was significant ($p<0.05$) (Table 2 and Fig. 2).

The mean vitamin A level was 14.3 mcg dL^{-1} in group 1 and 25.8 mcg dL^{-1} in group 2. The mean vitamin D level was 17.8 ng L^{-1} in group 1 and 26.2 ng L^{-1} in group 2. The mean vitamin E level was 8.2 µg mL^{-1} in group 1 and 11.5 µg mL^{-1} in group 2 and B12 was 102.4 pg mL^{-1} in group 1 and 436.8 pg mL^{-1} in group 2. The difference was significant ($p<0.05$) (Table 3).

No significant correlation was found among the serum level of vitamin except between vitamin B12 and vitamin E in group 1 (Table 4).

DISCUSSIONS

Recurrent aphthous stomatitis is one of the most prevalent oral mucosa diseases. It affects 5-25% of the population, mostly women and in higher socioeconomic groups[7,8].These idiopathic lesions are characterized by recurrent painful attacks. The condition is chronic and usually self-limiting in immunocompetent patients[9]. Etiology remains unclear but local trauma, emotional stress, allergy, toxin exposure, poor oral hygiene, vitamin deficiency and alterations in the oral flora are defined as risk factors[10,11]. The lesions are painful and their sizes range from 1 mm to a few centimeters in diameter. The lesion size is one of the diagnostic criteria used in classification, which is divided into 3 categories: Major, minor and herpetiform[12,13]. We performed this study to analyze vitamin A, D, E and B12 levels in serum among recurrent aphthous stomatitis patients and normal individual.

Our results showed that the most common site of occurrence of aphthous ulcers was labial mucosa in 26, buccal mucosa in 14, tongue in 10 and gingiva in 8 cases. Saha *et al*.[14] in their study RAS and blood levels of vitamins A, B12, D3 and E were correlated. The forty RAS patients and the forty healthy people were compared. Vitamins A, B12, D3 and E serum concentrations were evaluated in two groups using the ELISA technique. Vitamin A, B12, D3 and E blood levels were considerably lower in RAS patients than in healthy controls and there was a positive correlation between vitamin B12 and vitamin E levels in RAS patients.

We observed that the most common type of RAS was aphthous minor in 40, major in 11 and herpetiform in 7 cases. Nalbantoglu and Nalbantoglu[15] evaluated the vitamin D status in recurrent aphthous stomatitis in children. Seventy-two patients with minor recurrent aphthous stomatitis and 70 age-matched healthy controls was included in the study. Serum vitamin D levels were 16.4 (8.6) ng mL^{-1} in patient group and 23.1 (11.5) ng mL^{-1} in healthy controls. There was a statistically significant difference between the groups in terms of serum vitamin D levels. There was no significant correlation between serum vitamin D levels and the severity of the recurrent aphthous stomatitis. It was found that the mean vitamin A level was 14.3 mcg dL^{-1} in group 1 and 25.8 mcg dL^{-1} in group 2. The mean vitamin D level was 17.8 ng L^{-1} in group 1 and 26.2 ng L^{-1} in group 2. The mean vitamin E level was 8.2 µg mL^{-1} in group 1 and 11.5 µg mL^{-1} in group 2 and B12 was 102.4 pg mL^{-1} in group 1 and 436.8 pg mL^{-1} in group 2. No significant correlation was found among the serum level of vitamin except between vitamin B12 and vitamin E in group 1. Saral *et al*.[16] assessed the level of lipid peroxidation and status of antioxidant vitamins in patients with RAU. Thirty patients with RAU and 20 healthy controls were enrolled. Levels of vitamins A, E and C in both fluids were significantly lower ($p<0.05$ for vitamins A and E and $p<0.005$ for vitamin C, respectively) in patients with RAU than in healthy control subjects. Conversely, the levels of MDA in serum and saliva were significantly higher ($p<0.005$) in patients with RAU than in the control group. A strong and highly significant correlation was found between serum and salivary levels of vitamins A, E and C and MDA in patients with RAU.

CONCLUSION

Vitamin A, B12, D3 and E deficiencies have a substantial impact on RAS and there is a significant link between RAS patients' serum levels of B12 and E.

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