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Original Article

The role of hematologic parameters in recurrent aphthous stomatitis

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Abstract

Aim: Recurrent Aphthous Stomatitis (RAS) is one of the most common diseases of oral mucosa characterized by recurrent painful ulcers. Although many factors have been implicated in the etiology of the disease, it has not yet been fully elucidated. We aimed to investigate serum vitamin B_{12}, ferritin, folate, vitamin D, hemoglobin, and thyroid stimulating hormone (TSH) levels in patients with RAS.

Materials and Methods: The study involved in 60 patients (34 women, 26 men) with idiopathic RAS and 70 healthy volunteers (41 women, 29 men). The serum vitamin B_{12}, ferritin, folate, vitamin D, hemoglobin, and TSH levels of the patients were measured and statistically compared.

Results: Serum levels of vitamin B_{12} (204.5 vs. 301.0 pg/ml) and ferritin (31.0 vs. 48.5 ml/ng) were significantly lower and serum level of folate (7.7 vs. 6.7 ng/ml) were significantly higher in the patient group than control group ($p<0.05$). No significant difference was found between the number of patients and controls who both had abnormal levels of TSH (1.81±1.59 mU/L). There was no significant difference in serum levels of hemoglobin (14.64±1.88 gr/dL) and vitamin D (16.05±13.03 ng/ml) between the groups ($p>0.05$). No correlations were detected between RAS assessed by family history, mean duration of disease, and frequency of attacks and hematinic deficiencies.

Conclusion: Vitamin B_{12} and ferritin deficiency can play a role in the underlying etiology of RAS therefore we suggested the investigation of the vitamin B_{12} and ferritin status in RAS patients.

Keywords: Aphthous stomatitis; ferritin; folate; thyroid stimulating hormone; Vitamin B_{12}; Vitamin D

INTRODUCTION

Recurrent Aphthous Stomatitis (RAS) is a common disorder of the oral mucosa, affecting approximately 20-25% of the general population (1-3). Painful ulcers are solitary or multiple with a gray or yellow background, round or oval shape surrounded by an erythematous halo. RAS, generally occur in childhood or adulthood at first (4). Three main types are classified according to number, size, duration, location, and potential for scarring of ulcerations: Minor, major, and herpetiform aphthous stomatitis. RAS more commonly affects nonkeratinized or movable oral mucosa (i.e., buccal mucosa, labial mucosa, and tongue) (5). Etiology is unclear yet and a multifactorial etiopathogenesis with predisposing factors has been hypothesized. Genetics, microbial factors, immune-deficiency conditions, hematological deficiencies, malnutrition, endocrinological disorders, drugs, trauma, and stress have been suggested as etiological factors (6,7). There are many studies in the literature regarding the role of hematological deficiencies in RAS but the results are controversial. We aimed to investigate the levels of vitamin B_{12}, ferritin, folate, vitamin D, hemoglobin, and thyroid-stimulating hormone (TSH) levels in patients with RAS.

MATERIALS and METHODS

A prospective, controlled clinical trial was planned to investigate serum vitamin B_{12}, ferritin, folate, vitamin D, hemoglobin, and TSH levels in patients with RAS. The study was carried out in the University Hospital, Department of Dermatology. The study was approved by the ethics committee of Ataturk University (decision no: 2018/0017).

Sixty patients with RAS and 70 healthy individuals were included between June 2017 and April 2018 after giving

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their informed consent. We included the patients with oral ulcerations repetitive more than three times per year into the RAS group. Patients with a history of systemic disease, in particular, Behcet's syndrome and any other diseases with oral mucosal involvement, patients on a special diet, and patients with a history of usage of antibiotic, anti-inflammatory drug, and vitamin or antioxidant supplements for last 4 weeks were excluded from the study. The control group consisted of age and sex-matched healthy individuals. The following laboratory tests were performed: liver and kidney function tests, complete blood count, vitamin $B_{12,}$ ferritin, vitamin D, folate, and TSH.

Statistical analysis

Statistical analysis was evaluated using SPSS software, version 22. Descriptive data were shown as n, % in categorical data, and as median interquartile range (25-75 percentile values) in scale data. Chi-square test was used to compare categorical data. The normality of data was tested by using a Kolmogorov–Smirnov test. Mann–Whitney U-test and Kruskal Wallis test was used for not have a normal distribution. A P-value of less than 0.05 was considered statistically significant.

RESULTS

Sixty patients (26 male and 34 female) with a mean age of 31.0 ± 11.1 years and 70 healthy individuals (29 male and 41 female) with a mean age of 36.1 ± 16.3 years were included in this study. There was no significant difference in age or gender between the patient and control groups (Table 1). Detailed features of patients are specified in Table 2.

Table 1. Demographic characteristics of groups

		Patients		Controls		p[a]
		n	%	n	%	
Age	≤30 years	35	58.3	37	52.9	0.531
	>30 years	25	41.7	33	47.1	
Gender	Female	34	56.7	41	58.6	0.827
	Male	26	43.3	29	41.4	
Job	Student	20	33.3	22	31.4	0.692
	Other	25	41.7	34	48.6	

[a] Chi square test

1. Hematinic deficiencies and TSH in patients compared with healthy controls

Serum levels of vitamin B_{12} ($p<0.001$) and ferritin ($p<0.002$) were significantly lower in the RAS patients than the controls (Table 3). Vitamin B_{12} deficiency (<200 pg/ml) and ferritin deficiency (<23.9 ml/ng for men, <11 ml/ng for women) were found significantly higher between the patient and control groups (Figure 1).

Serum levels of TSH ($p<0.007$) were significantly lower in the RAS patients than the controls (Table 3) but no significant difference was found between the number of the patients and controls who had abnormal levels of TSH (<0.34, >5.6 mU/L).

Table 2. Detailed features of patients

		n	%
Place of residence	urban	52	86.6
	rural	8	13.4
Family history	positive (+)	32	53.3
	negative (-)	28	46.7
Mean duration of disease (year)	<5	28	46.7
	≥5	32	53.3
Frequency of attacks (per year)	<10	16	26.7
	10-19	10	16.6
	20-29	21	35.0
	≥30	13	21.7
Presence of ulcer during examination	positive (+)	51	85.0
	negative (-)	9	15.0

Table 3. The respective statistical analysis of hematinic parameters in RAS patients and controls

	Patients		Controls		p[a]
	Median	IQR	Median	IQR	
Vitamin B_{12} (pg/ml)	204.5	170.5-280.0	301.0	232.0-398.0	<0.001
Ferritin (ml/ng)	31.0	9.4-67.3	48.5	23.9-117.0	0.002
Folate (ng/ml)	7.7	6.0-10.0	6.7	5.3-8.3	0.014
Vitamin D (ng/ml)	11.0	8.9-18.8	17.2	9.6-23.8	0.056
Hemoglobin (gr/dL)	14.8	13.3-16.0	15.1	13.9-16.1	0.185
TSH (mU/L)	1.5	0.8-2.1	2.0	1.4-2.9	0.007

[a] Mann-Whitney U test, IQR: Interquartile Range

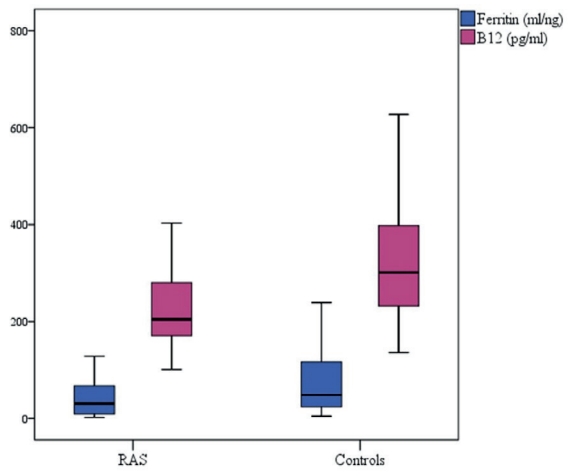

Figure 1. Vitamin B_{12} and ferritin levels of patient and control groups

The serum level of folate (p<0.014) was significantly higher in the RAS patients than the controls (Table 3). No significant difference was detected in folate deficiency (<3.1 ng/ml) between the patient and control groups.

No significant difference was detected in serum levels of hemoglobin and vitamin D between the groups (Table 3). Similarly no significant difference was detected in hemoglobin deficiency (<13 g/dl for male, <12 g/dl for female) and vitamin D deficiency (<25 ng/ml) between two groups.

2. Hematinic deficiencies and TSH in RAS patients according to gender, age and living area

Statistically significant differences in the levels of ferritin and hemoglobin (p<0.001 for both) were found between male and female RAS patients (Table 4). Serum levels of ferritin and hemoglobin were lower for female patients than those for male patients (Figure 2). There was no significant difference between gender and other hematinic parameters.

Vitamin B_{12} level was lower (p<0.006) for young patients (≤30 years) than that for old patients (>30 years) (Table 4, Figure 3). There was no significant difference between age and other hematinic parameters.

Vitamin B_{12} level (p<0.049) was higher for the urban patients than that for rural patients (Table 4, Figure 3). No significant difference was detected between the rural and urban patients and the controls for other hematinic parameters.

Table 4. Hematinic deficiencies in RAS patients according to gender, age and living area

		Mean	p[a]
Ferritin (ml/ng)	Female	9.9 (5.7-19.0)	<0.001
	Male	67.3 (42.0-109.0)	
Hemoglobin (gr/dL)	Female	13.4 (12.6-14.4)	<0.001
	Male	16.1 (15.6-17.0)	
Vitamin B_{12} (pg/ml)	≤30 years	190.0 (150.0-259.0)	0.006
	>30 years	227.0 (200.0-354.0)	
Vitamin B_{12} (pg/ml)	Urban	214.5 (180.0-294.5)	0.049
	Rural	163.5 (131.0-213.0)	

[a] Mann-Whitney U test

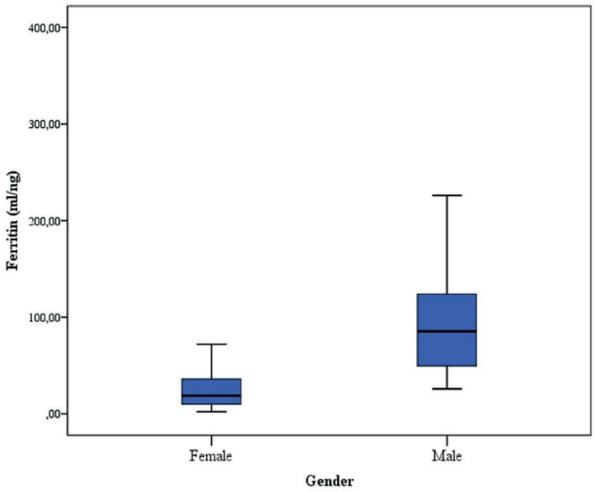

Figure 2. Ferritin levels of female and male RAS patients

Table 5. Hematinic deficiencies in RAS patients according to characteristics of disease

		Vitamin B_{12} (pg/ml)	Ferritin (ml/ng)	Vitamin D (ng/ml)	Folate (ng/ml)	Hemoglobin (gr/dL)	TSH (mU / L)
Family history [a]	+	201.0 (170.0-271.5)	36.0 (10.0-67.3)	15.0 (9.0-20.5)	7.6 (6.0-10.0)	14.9 (13.4-16.1)	1.7 (0.8-2.2)
	-	210.08 (172.0-290.5)	23.5 (7.3-61.5)	10.5 (8.2-14.7)	7.7 (6.3-11.2)	14.4 (13.3-16.0)	1.5 (1.3-2.0)
Mean duration of disease (year) [a]	<5	221.5 (148.5-329.5)	30.0 (9.4-49.5)	15.0 (9.4-19.59	7.6 (6.0-10.7)	14.2 (13.4-15.6)	1.7 (1.1-2.1)
	≥5	199.5 (175.0-245.5)	31.5 (9.4-90.4)	10.5 (8.0-18.3)	7.9 (6.2-10.0)	15.0 (13.3-16.2)	1.5 (0.8-2.2)
Frequency of attacks (per year) [b]	≤12	219.0 (176.0-243.0)	35.5 (13.5-80.3)	11.0 (8.2-16.9)	8.3 (6.9-11.9)	14.0 (13.1-16.2)	1.8 (1.1-2.2)
	13-24	209.0 (190.0-403.0)	56.0 (6.6-116.0)	9.0 (6.5-10.0)	8.1 (7.4-10.7)	15.0 (14.0-16.0)	1.5 (0.7-1.9)
	25-36	207.0 (155.0-284.0)	16.0 (8.1-54.0)	11.0 (10.0-24.0)	7.4 (5.6-9.5)	14.6 (13.4-15.9)	1.5 (0.8-2.1)
	>36	194.0 (187.0-276.0)	31.0 (9.0-40.0)	14.9 (10.0-19.0)	6.5 (6.0-9.8)	15.2 (13.3-16.0)	1.9 (1.4-2.7)
Presence of ulcer during examination [a]	+	199.0 (170.0-276.0)	31.0 (9.0-69.6)	11.0 (8.9-18.5)	7.6 (6.0-10.0)	14.6 (13.3-16.0)	1.5 (1.0-2.0)
	-	216.0 (200.0-379.0)	32.0 (12.2-42.0)	11.0 (8.3-19.0)	8.0 (7.0-8.6)	14.9 (13.5-16.2)	1.8 (0.8-2.6)

[a] Mann-Whitney U test, [b] Kruskal Wallis Test

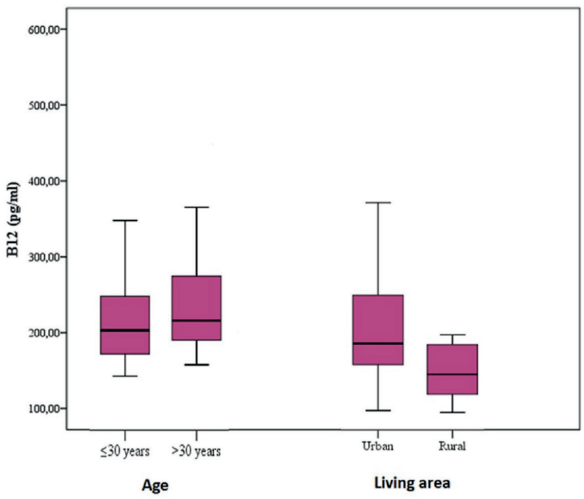

Figure 3. Vitamin B_{12} levels of patients

3. Hematinic deficiencies and TSH in RAS patients according to characteristics of disease

As the duration of the disease and the number of attacks increased vitamin B_{12} level decreased. It was noticeably lower for patients with family history and those with the presence of ulcer during the examination. No significant difference was detected by the RAS assessed by family history, mean duration of disease, frequency of attacks, the presence of ulcer during the examination, and hematinic deficiencies and TSH (Table 5).

DISCUSSION

RAS is the most common disorder of oral mucosa with recurrent painful ulcerations. The prevalence of the disease varies from 5% to 60%, with an average of 20% (5,8,9). Although RAS can affect people of any age, oral ulcerative episodes first appear before the age of 30 years in about 80% of patients. Usually, the severity and frequency decrease as age advances (10,11). In our study, first oral ulcerative episodes have appeared before the age of 30 years in the patients of 78.8%. Approximately 40% of the patients with RAS have a family history (12,13). 53.3% of the patients have a family history in our study.

Disease affecting both sexes is more common in women and especially in groups with high socioeconomic levels, in individuals living in stressful environments such as soldiers and students (8,14) In our study, 56.7% of the patients were women and 33.3% of patients were students.

The etiology of RAS has not been fully understood yet, in addition to genetic factors, emotional stress, microbial agents, trauma, diet, hormonal changes, drugs, immunological factors, atopy, nutritional and hematological deficiency are considered as predisposing conditions (15).

There is a general view that serum iron, ferritin, zinc, folate, vitamins B1, B2, B6, and B_{12} deficiency have a role in the etiopathogenesis of aphthous stomatitis but none of them have been proven precisely. And also mechanisms by which vitamin deficiencies affect RAS are not well understood. Sun et al. reported that there is a significant relationship between iron, vitamin B_{12}, and folate deficiencies and RAS, that these deficiencies may trigger oral ulcers by causing epithelial atrophy (16). Folic acid and vitamin B_{12} are essential for the synthesis of DNA and cell division. Their deficiencies affect oral mucosal cells which have a high turnover rate. Björkegren et al. reported that the findings of cheilitis and mucosal atrophy of the tongue were thus the only significant clinical findings of abnormal serum levels of folate (17).

We found vitamin B_{12} deficiency (36.6% vs. 11.4%) and ferritin deficiency (31.6% vs. %7.1) significantly higher between the patient and control groups. Folate deficiency was detected in one patient and one control in this study. It is commonly accepted that there is a relationship between RAS and vitamin B_{12} deficiency, and also it has been considered by some authors that aphthae regress after vitamin B_{12} treatment regardless of the serum levels of vitamin B_{12}. In a study by Volkov et al., they showed that %74.1 of RAS patients had been treated successfully with using once-daily sublingual vitamin B_{12} for six months (18). Ślebioda et al. indicated an association between iron and vitamin B_{12} deficiency and RAS in a Polish population. Iron replacement in RAS patients with ferritin or iron deficiency may be beneficial, even if the patients do not have iron deficiency anemia (19).

Wray et al. investigated vitamin B_{12}, iron, and folic acid deficiency in patients with RAS. They found a high incidence of hematinic deficiencies and good response to replacement therapy so they suggested hematological screening of such patients (20). Lopez-Jornet et al. investigated the deficiency of vitamin B_{12}, iron, ferritin, and folic acid in a study conducted with 186 patients, 14.1% of the patients were detected hematinic deficiency and the difference was significantly higher compared with the controls (21). In another study conducted with 517 RAS patients and 187 healthy individuals, it was reported that vitamin B_{12} deficiency was significantly higher in the patient group than the control group and no significant difference was detected in folate and ferritin levels (9). Pişkin et al. investigated serum levels of vitamin B_{12}, iron, ferritin, folic acid and only vitamin B_{12} deficiency was significantly higher in the patients with RAS compared to the control group (22).

Hemoglobin deficiency was found 8.3% in the patients and 2.9% in the controls in our study and no significant difference was detected between the groups. Similarly, Al-amad et al. reported that no significant difference was detected in Hb deficiency between the RAS patients and controls (23). However, Sari et al. reported that hemoglobin deficiency was found higher in patients compared to the control group (24). Although we detected the highest number of vitamin D deficiencies (88.3%) among the hematinic parameters in the patients but there was no significant difference compared with the controls in this study.

And also, there was no correlation between the frequency of ulcers and serum levels of vitamin D. Studies with different results have been reported on the relationship between vitamin D deficiency and RAS (23,25,26). Al-Amad et al. reported in a recent study that vitamin D deficiency plays a role in aggravating oral ulcerative episodes but not in their onset (23). Krawiecka et al. conducted a study with 66 patients found no significant difference between the patients with RAS and controls in serum levels of vitamin D and no correlation between severity of disease and vitamin D (25). Nalbantoğlu et al. reported the first study conducted within the pediatric population with RAS, as a result of the study serum levels of vitamin D were significantly lower in the patients than controls but there was no correlation between the severity of the RAS and vitamin D levels (26).

In our study, there was no significant difference between the patient and control groups who had abnormal levels of TSH. As we examined the abnormal TSH levels, 1 patient had hyperthyroidism, 1 control had hypothyroidism and 1 control had hypothyroidism. Özdemir et al. investigated levels of TSH, free and total thyroxine (fT4, TT4), free and total triiodothyronine (fT3, TT3), thyroglobulin, antithyroid peroxidase, and antithyroglobulin antibody. The frequency of thyroid autoimmune-related disorders was detected higher in RAS patients. Although the patients had lower fT4 and higher fT3 and their TSH increased but they are not significantly different than the controls in the study (27).

CONCLUSION

Our results were presented partial data about the vitamin B_{12}, ferritin, folic acid, hemoglobin, vitamin D, and TSH status of the Turkish people. In conclusion, data suggest linkage of serum levels of vitamin B_{12} and ferritin in RAS patients, and replacement would be beneficial in the treatment. As for the relationship between thyroid disorders and RAS, further studies are needed to answer 'if it is necessary to investigate thyroid diseases in patients with RAS'. The limitation of our study was being performed in a single center. Hematinic deficiencies may vary with socioeconomic status, nutritional habits, and genetic structure of societies. Therefore, multicenter studies involving more populations are needed.

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