

Evaluation of Hematologic Status in Patients with Recurrent Aphthous Stomatitis in an Iranian Population

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Article information	Abstract
<p>Article history: Received: 30 Jan 2013 Accepted: 6 Feb 2013 Available online: 28 Apr 2013 ZJRMS 2014 July; 16(7): 21-25</p> <p>Keywords: Aphthous Stomatitis Hematologic Status Oral Ulcer</p> <p>*Corresponding author at: Department of oral Medicine, School of Dentistry, Zahedan University of Medical Science, Zahedan, Iran. E-mail:nosratzehi@yahoo.com</p>	<p>Background: The aim of the present study was to compare hematologic problems in patients with recurrent aphthous stomatitis, with a control group.</p> <p>Materials and Methods: In this cross sectional study, 30 subjects with recurrent aphthous stomatitis and 30 healthy individuals were included as the case and control groups, respectively. After diagnosis was established a 10 ml sample of the subjects' blood was used to determine serum levels of iron, ferritin, vitamin B12, folic acid and zinc in each subject. Independent <i>t</i>-test was used to analyze data.</p> <p>Results: The average serum iron, serum ferritin, vitamin B12, folic acid and serum zinc levels in the case and control groups were assessment, demonstrating no statistically significant differences between the two groups ($p>0.05$).</p> <p>Conclusion: According to the results of the present study, hematologic deficiencies cannot play a role in etiology of aphthous stomatitis.</p>

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Introduction

Recurrent aphthous stomatitis is one of the most common ulcerative lesions of the oral cavity with a prevalence rate of approximately 5-66% [1] in the general population and an average rate of 20% [1, 2] all over the world. The most common age reported for such lesions is 10-19 and it is generally more common in females than in males. Several theories have been suggested for the etiology of the lesions, including genetics, blood deficiencies, allergy, nutritional factors, hormonal disorders, trauma, and stress [3].

Factors involved in the disruption of mucous membranes in patients with aphthous stomatitis are classified into three groups: primary immune regulation disorder, compromise of mucous barriers, and increased contact with an antigen. Several factors are involved in the compromise of mucous barriers, resulting in increased recurrence rate. Nutritional disorders, such as lack of vitamin B12, iron etc not only influence the hematologic system but also may affect the proliferation of the oral mucosa resulting in a decrease in the relative thickness of the oral mucosa [1]. Mucous barrier is effective in preventing aphthous lesions, which explains why aphthous stomatitis is usually seen in non-keratinized mucosa [1].

The prevalence of blood deficiencies in patients with aphthous stomatitis is 18-28% [1] and different studies have reported various blood deficiencies in different societies [5-9]. In some studies no differences have been

observed in iron, ferritin and folic acid levels between patients with aphthous stomatitis and the controls and simply vitamin B12 deficiency has been reported [5, 6]. In some studies no recurrence of aphthous lesions has been observed subsequent to replacement therapy [9, 10].

Vitamin B12 (cobalamin) and folic acid are necessary for creation and growth of red blood cells in the bone marrow. Folic acid is required for enzymatic reactions of purine, pyrimidine, RNA, DNA, and thus, to build proteins. Folic acid is found in fruits and vegetables. It will not be stored in large quantities in the body. Thus, the existence of a continuous supply of this vitamin in the diet is required. Vitamin B12 can be stored for several years, and nutritional deficiencies are rare and usually do not happen until late adulthood [11]. Deficiency anemia, particularly iron deficiency, vitamin B12 deficiency and folic acid deficiency, cause occurrence of recurrent aphthous stomatitis [3]. Vitamin B12 and folic acid are the micronutrients required for the proper functioning of the immune system. The deficiency of these two factors leads to the impaired immune function; apoptosis of ancestral cells in bone marrow and the presence of leukocytes with hypomethylated DNA in the peripheral blood circulation [12].

Folic acid plays a crucial role in the synthesis of DNA and proteins, so any mechanism in which cell proliferation is involved, could be affected by this micronutrient deficiency, such as cell-related immunity

which is seriously affected in folate deficiency. Folic acid plays an important role in preventing from chromosome degradation and loss of DNA methylation and on the other hand, reduction of levels of folic acid is and vitamin B12 is associated with increased levels of homocysteine in the blood. Homocysteine is the strong stimulus for T-cells, which induces cell activity and cell differentiation along with activated-induced cell death and apoptosis. Homocysteine has various effects on function of immune system, blood circulation and tissues. B12 and folic acid deficiency associated with increased homocysteine levels is usually seen in patients with increased cellular immune activity such as Alzheimer's disease and rheumatoid arthritis [13].

Various studies have been conducted concerning whether all patients must be examined for blood deficiencies, reporting different results. Some studies have reported that these tests are necessary for the assessment of all the patients [5, 6], and some have observed no significant differences in these factors between patients and controls [5]. Since oral aphthous lesions, especially large lesions, disrupt the daily routine of patients and might lead to cancerphobia, finding the reason for and the origin of such ulcers and presentation of effective treatment modalities seem necessary. Therefore, considering the various etiologic factors suggested in this regard, we decided to analyze blood factors to evaluate the hidden deficiencies of blood elements, such as iron, folate, vitamin B12 and zinc because replacement of these elements results in the rapid improvement of the ulcers in some cases [8, 9]. Since no studies had been carried out on the subject in Zahedan, we decided to evaluate the serum levels of iron, ferritin and zinc in patients with recurrent oral aphthous lesions referring to the department of oral medicine, faculty of dentistry at Zahedan University of Medical Sciences in 2007-2008.

Materials and Methods

In this analytical cross-sectional study, which was approved by the Ethics Committee of Zahedan Medical Sciences University, the cases were selected from the patients referring to the Department of Oral Medicine, Faculty of Dentistry at Zahedan University of Medical Sciences between October 2009 and March 2010.

Thirty patients with oral aphthous ulcers consisting of 13 males and 17 females with an average age of 29 ± 7.3 years and 30 healthy individuals from those referring to the Faculty for other reasons were selected as controls; age, sex, and socioeconomic status of the subjects were matched. All the selected patients suffering from oral aphthous lesions were examined by an oral medicine specialist.

Diagnosis was based on the presence of round symmetrical yellowish white ulcers less than 1 cm in diameter with an erythematous halo covered with a detachable membrane, which recovered without any scars. A questionnaire was provided and filled with precision for the assessment of all the patients. Conditions required for

being included in the study were:

1. Presence of recurrent aphthous lesions;
2. Absence of systemic conditions, including Reiter's syndrome, Behcet's syndrome, Crohn's disease and gastrointestinal problems;
3. No consumption of folic acid, vitamin B12, iron and zinc in drug form and other supplement during the past 8 weeks.

Pregnant women and those who avoided signing the consent form were excluded from the study.

After clinical tests and establishing diagnosis of aphthous lesions and signing a written consent form, the patients were referred to the Department of Oral Medicine for paraclinical tests, including serum levels of iron, zinc, vitamin B12, folic acid and ferritin. The assistant disinfected each patient's elbow to obtain a 10 ml sample of their venous blood using a 10 ml disposable sterile syringe and a 27-G needle (Supa, Tehran, Iran). The blood sample was poured into two test tubes. Then the blood samples were transferred to the laboratory within an hour. After coagulation stages and separating the clot from the samples, Biotech Centrifuge System was used to separate serum from the clot at a speed of 7000 rpm within 25 minutes and was transferred to transparent 5 ml plastic pipes. Samples were kept for 1 month in the laboratory refrigerator at -20°C ; the samples were then delivered to refrigerator at -70°C in the Clinical Researches Center of Imam Ali Hospital.

For the study of the serum level of iron, Biochemical Kit (Iranian) with the serial no. of 11-514 was used manually. For the study of the serum level of ferritin, Padtan Elm Kit (Iranian) with the serial no. of Fr0108 was used by RIA (Radio Immune Assay), and for the study of the serum level of Zinc, Randox Kit (England) with the serial no. of 2332 was used. Vit B12 and folic acid were evaluated by Elexis 2016 and electroluminasance method.

The results were compared to the normal values of the manufacturing company. For statistical analysis, SPSS-17 software and independent *t*-test were used.

Results

In the present study 30 patients with recurrent aphthous lesions, consisting of 17 females (43.3%) and 13 males (56.6%) with an average age of 31 ± 7.3 years and 30 healthy individuals, consisting of 17 females and 13 males with an average age of 33 ± 9.8 years were included. There was no significant difference between the two groups regarding the age of the subjects.

A total of 56.6% of the lesions were found in mandibular vestibule and 33.3% of the lesions were seen in the ventral aspect of the tongue, which were the most common sites of the lesions in this study. The mean serum levels of iron, ferritin, vitamin B12, folic acid and zinc in the case and control groups are presented in the table 1.

Mean serum levels of ferritin were 69.79 and 87.82 ng/dL in the case and control groups, respectively, demonstrating a higher mean of ferritin than the normal level in both groups; however, there was no statistically

significant difference between the two groups.

The mean serum levels of zinc were 117.11 and 118.07 mg/dl in the case and control groups, respectively, demonstrating a higher mean than the normal level in both groups; however, there was no statistically significant difference between the two groups.

The mean serum levels of iron were 90.92 and 107.84 mg/dL in the case and control groups, respectively, demonstrating a higher mean than the normal level in both groups; however, there was no statistically significant difference between the two groups.

The mean serum levels of vitamin B12 were 292.8 pg/ml and 317.7 pg/ml in the case and control groups respectively there was no statistically significant difference between the two groups.

The mean serum levels of folic acid were 10.0 ng/ml and 12.6 ng/ml in the case and control groups respectively there was no statistically significant difference between the two groups.

Table 1. Serum ferritin, serum iron and serum zinc levels in patients with recurrent aphthous stomatitis compared to control group

	Groups	Mean ±SD	p-Value
Serum iron level (mg/dl)	Patient	92.92±31.80	0.09
	Control	107.84±39.27	
Serum ferritin level (ng/dl)	Patient	69.79±54.76	0.36
	Control	87.82±83.92	
Serum zinc level (mg/dl)	Patient	117.11±23.52	0.91
	Control	118.07±41.45	
Serum vitamin B12 (pg/ml)	Patient	314.7±141.4	0.05
	Control	292.8±97.4	
Serum folic acid (ng/ml)	Patient	10.9±5.19	0.17
	Control	12.6±4.1	

Discussion

The present study was carried out to evaluate the serum levels of iron, ferritin and zinc in patients with recurrent aphthous stomatitis referring to the department of oral Medicine, Faculty of Dentistry at Zahedan University of Medical Sciences in 2007-2008.

Discomfort in patients with recurrent aphthous stomatitis is relatively high. The patients' life quality has been affected by painfulness of these mucosal lesions. Canker sores increase the flow of saliva, and interfere with eating, drinking and speaking. Since the etiology of RAS is unknown, many patients are receiving medication only to relieve pain [5].

Patients with recurrent aphthous stomatitis in the present study were mostly women and the peak age for the condition was in the 3rd and 4th decade of life, which is consistent with previous reports [4, 14].

Different studies have reported various deficiencies of blood elements in different societies [5-9]; some of these studies have not reported any decreases in iron, ferritin, and folic acid levels in patients with recurrent aphthous stomatitis compared to controls and have only reported vitamin B12 deficiency [5, 6]. In the present study, no statistically significant differences were observed in the serum levels of ferritin, iron, and zinc between the two groups. However, in a study carried out by Barnadas et al. in 1997 the iron, ferritin, vitamin B12 and folic acid levels

were low in 18% of the subjects; 5% had iron deficiency; 12% had folic acid deficiency and 5% had vitamin B12 deficiency, demonstrating statistically significant differences between the case and control groups [15].

In a study carried out by Ogura et al. in 2001 on 118 patients with aphthous stomatitis, who had more than 4 recurrences each year, and a control group of 118 healthy individuals it was concluded that aphthous patients not only have low serum levels of iron and vitamin B₁, but there is also decreased serum levels of calcium and vitamin C in such patients [8]. Piskin et al. discovered that there is no significant difference in the serum levels of iron, ferritin and folic acid between the case and control groups [5], which is consistent with the results of our study in the case of iron and ferritin serum levels.

In a study carried out by Scully et al. a relationship was observed between folic acid, iron and vitamins B₁, B₂, B₆, and B₁₂ deficiency in 18-28% of classic cases of aphthous stomatitis compared to 8% in healthy individuals [4].

Koyabsi et al. in 2006 reported that there is no significant difference between deficiency of folic acid, Ca, Mg, P, Fe, TIBC and ferritin between the case and control groups [6], which is consistent with the results of the present study in the case of iron and ferritin serum levels.

In a study carried out by Burgan et al., although most of the aphthous patients were reported to have reduced levels of blood hemoglobin, there were no statistically significant differences with the control group [16].

The studies we are to mention next have reported different results compared to our results.

In a study carried out by Burgan et al. in 2006 on 54 oral aphthous patients, it was demonstrated that 37.8% of the subjects had reduced serum level of ferritin, which was statistically significant compared to 18.2% of the controls. In this study, 4.9% of the subjects had folate deficiency, though none of the controls had such a deficiency. A total of 26.6% of the subjects in the case group and 12.6% of the controls had vitamin B12 deficiency, demonstrating statistically differences [15].

Khademi et al. in 2006 reported that mean serum level of zinc in the case group was 70±9 mg/dl, compared with 94±14 mg/dl in the controls, demonstrating a statistically significant difference. These results are not consistent with the results of the present study [17].

Solak Tekin et al. demonstrated that 30% of aphthous patients suffered from anemia but only 10.9% of the controls had anemia. A total of 61.8% of the cases and 37% of the controls were reported to have other blood deficiencies, demonstrating a statistically significant difference. In the case group 25.4% had ferritin deficiency and 26.3% had vitamin B12 deficiency and only one patient had folic acid deficiency; however, in the control group only 5.4% had ferritin deficiency and only 5.4% had vitamin B12 deficiency with no subjects having folic acid deficiency. Levels of hemoglobin and hematocrit, serum iron, and ferritin in the case group were significantly less than those in the control group; however, iron-binding capacity of the serum was high in the case group [18].

In this study, there was no statistically significant difference between the serum levels of vitamin B12 in patients and control group, which was consistent with the study of Olson et al. [19] and Thongprasom et al. [7] and inconsistent with the study of Piskin et al. [5], Koybasi et al. [6], Burgan et al. [16], Volkov et al. [20] and Scott et al. [21]. In the present study there was no statistically significant difference between serum levels of folic acid in patients and control groups; which was consistent with the study of Olson et al. [19], Piskin et al. [5], Volkov et al. [20] and Tekin et al. [18] and inconsistent with the study of Thongprasom et al. [7], Koybasi et al. [6], Burgan et al. [16], and Scott et al. [21].

According to the information obtained from the examination of patients, 3 out of 12 male patients had vitamin B12 deficiency (i.e. 25% of men); while 2 out of 18 female patients had vitamin B12 deficiency (i.e. 1/9 of women), and this could indicate that vitamin B12 deficiency in men could be more related to the outbreaks of RAS. However, in all subjects in the healthy group were in the normal range and no B12 deficiency was observed. Another implication of these results is that the prevalence of vitamin B12 deficiency is higher among men in the community.

Vitamin B12 deficiency impairs the metabolism of folate which can cause folic acid deficiency. Therefore, it is also recommended to measure folic acid in patients with vitamin B12 deficiency, because vitamin B12 highly contributes to normal function of the neuropsychological system and disorders such as anxiety and depression are considered as risk factors for RAS [13].

Previous studies have discussed the possible role of folic acid in the aetiology of RAS [9]. However, some other studies have rejected this relationship [19]. It has been tried to find a correlation between vitamin B12 and folic acid with RAS, that no relationship was observed, and this may be partly influenced by geographic and

nutritional factors of the region, and that RAS is seen in higher socioeconomic classes. Thus, it is likely that normality of levels of vitamin B12 and folic acid is due to the more appropriate nutritional status of the group. However, it is recommended that studies should be conducted on groups with larger sample size and another role of vitamins and minerals in patients with RAS should be investigated and also supplementary methods to measure folic acid in RBC should be used in order to examine the relationship between this vitamin and RAS more closely.

According to the results of the present study there are no statistically significant differences in the serum levels of iron, zinc, ferritin, vitamin B12 and folic acid between patients with recurrent aphthous stomatitis and healthy individuals. so it is not necessary to evaluate the level of blood factors such as serum levels of vitamin B12, folic acid, iron, zinc and ferritin in patients with RAS, unless in cases where there is any evidence of anemia, digestive problems and nutritional deficiencies.

However, it is suggested that further studies be carried out on bigger sample sizes to evaluate the role of other vitamins and minerals in these patients.

Acknowledgements

This study is extracted from two general dentistry thesis, with the codes 456 and 527.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Zahedan University of Medical Science.

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Please cite this article as: Arbabi-Kalati F, Nosratzahi T, Sarabadani J, Niazi A. Evaluation of hematologic status in patients with recurrent aphthous stomatitis in an Iranian population. *Zahedan J Res Med Sci.* 2014; 16(7): 21-25.