Hematological study of patients with recurrent aphthous stomatitis - Case control study

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Background and Objectives: Recurrent aphthous stomatitis is common oral condition, characterized by multiple, recurrent, small, round, or ovoid ulcers with circumscribed, erythematous haloes, and yellow or grey floors. Its etiology and pathogenesis is not entirely clear linked to systemic diseases and among these, anemia, iron deficiency, folic- acid, and vitamin B12 deficiencies. The aim of this study to investigate the association between recurrent aphthous stomatitis and hemoglobin, serum ferritin, folic acid, and vitamin B_{12} deficiency.

Patient and Methods: This prospective cross sectional case control clinical study was conducted on 50 patients with recurrent aphthous stomatitis. The control group composed of 50 healthy age and sex-matched subject. The patient demography about family history of recurrent aphthous stomatitis, and smoking was recorded, also the hemoglobin, serum levels of ferritin, folic acid, and vitamin B_{12} has been investigated.

Results: The low hemoglobin was reported in 30% and vitamin B 12 deficiency in 31% of the patient so low hemoglobin and B_{12} deficiency have significant correlation with recurrent aphthous stomatitis. Patients with low hemoglobin, vitamin B_{12} deficiency , positive family history, and non smoker have been found to have the highest risk for having recurrent aphthous stomatitis.

Conclusion: Recurrent aphthous stomatitis is a multifactorial disease. Positive family history, low hemoglobin level , vitamin B12 deficiency, and nonsmoking status are among the important predisposing factors. Routine hematological screening and tests for iron status, folic acid, and vitamin B12 status should be assessed in all patients with recurrent aphthous stomatitis.

Key words: hemoglobin, ferritin, folic acid, vitamin B_{12}, recurrent aphthous stomatitis.

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Introduction

Recurrent aphthous stomatitis (RAS) is an inflammatory condition of unknown etiology characterized by painful recurrent, solitary or multiple ulcerations of the oral mucosa. (1) It is one of the most common oral diseases worldwide. The prevalence ranges from 2% to 66% in different populations, (2) with a high prevalence among women. (3) They are usually painful, shallow round ulcers with an erythematous halo covered by a yellowish gray fibro membranous layer. (4)

The clinical features of RAS consist of recurrent bouts of one or several, shallow, ovoid, painful ulcers, occurring at intervals of a few days or up to 2-3 months. Three clinical presentations of RAS are recognized: minor recurrent aphthous stomatitis (MiRAS), major recurtent aphthous stomatitis (MjRAS); and herpetiform ulceration (HU). Patients may sometimes present with a mixed pattern of RAS but this is relatively uncommon. (5)

Although it's etiology remains unclear, recurrent aphthous has been hypothesized as having a multifactorial aetiopathogenesis, as an interaction between predisposing factors

(e.g. gender, age, family tendency, smoking habits, stress, hormone imbalance) and/or systemic conditions and immunological components in genetically predisposed subjects.[3, 6,7] RAS may be associated with several diseases such as Behcet disease, gluten-sensitive enteropathy, pernicious anemia, cyclic neutropenia, inflammatory bowel disease, and FAPA (periodic fever, aphthous stomatitis, pharyngitis, and adenitis).[8-12] Considering the systemic conditions, recurrent aphthous ulcer may also be secondary to haematinic deficiencies. The role and prevalence of deficiencies of haematinics such as iron, folic acid, vitamin B_{12}in the aetiopathogenesis of recurrent aphthous is not well known and many contradictory results have been reported. [7, 13-17]

The diagnosis of RAS rests mainly on two features: a history of recurrent ulcers and the presence of the typical solitary or multiple round or ovoid ulcers on labial mucosa on clinical examination. [18] Several studies have suggested the importance of iron, folic acid and vitamin B_{12} deficiencies and nutritional intolerance, but some controversial results have been reported. [15,19,20] It is important to consider a possible systemic cause, particularly when adults suddenly develop what appears to be RAS. It is common practice therefore, to assess the full (complete) blood cell count, red cell folate, and serum levels of ferritin (or equivalents) and vitamin B_{12}, which may also show potential gastrointestinal disease, but, in the absence of other manifestations, screening for gluten sensitive enteropathy is usually fruitless. [10]

Nutritional deficiencies or hemotologic diseases have been documented in 20% of patients with RAS. [21, 22] The prevalence of iron, folic acid, and vitamin B12 deficiencies and their role in the pathophysiology of RAS are not well known, though several reports have suggested their importance and relevance. [10, 23-28]

Porter *et al*,[29] founded ferritin levels to be low in 11.6% of their patients with RAS, while the vitamin B_{12} levels were low in 3.2%. The ferritin concentrations were significantly lower than in the controls. It has been suggested that possible vitamin B_{12} deficiency should be considered in all subjects with RAS. Another study which was done by Piskin, *et al* in 2002 found that the serum iron levels were below normal in six RAS subjects (four males, two females) and one control (male) ($P > 0.05$). Ferritin levels were low in six RAS subjects (one male, five females) and three controls (all females) ($P > 0.05$). Serum folic acid levels were below normal values in four RAS subjects (three males, one female) ($P > 0.05$) and vitamin B_{12} levels were lower than normal in eight RAS subjects (two males, six females) ($P<0.05$). Folic acid and vitamin B_{12} levels were within normal limits in all control subjects.[10] In the study done by Lopez *et al* in 2014, they observed a greater presence of hematinic deficiencies in patients with aphthae versus controls, though statistically significant differences were not recorded. The frequency of hematinic deficiencies was 14.14% in patients with recurrent aphthous versus 6.39% in controls (P = 0.086). Iron, folate, and vitamin B_{12} play an important part in RAS, though the subject remains open to controversy. [30]

Regarding family history of RAS, a genetic predisposition for the development of aphthous ulcer is strongly suggested, as in one study about 40% of patients have a family history and these individuals develop ulcers earlier and are of more severe nature. [31] A number of several other studies have shown a familiar trend in the development of RAU [32] and the correlation is also greater in identical twins [33], demonstrating the existence of a genetic influence in the episodes. High incidence of aphthous stomatitis was reported by Miller *et al* [12] in identical twins compared with nonidentical twins (90% vs 60%).

Some researchers thought that smoking has protective effect and this protective effect is related to the increased keratinization of the oral mucosa in smokers and that this keratin layer acts as a mechanical and chemical barrier against trauma or microbes. [34] Few investigators suggested that smokers may be less psychologically stressed than non-smokers and that some psychological trigger might affect RAU development. [35]

The aim of this study was to investigate the association of Hb, serum ferritin, folic acid,

and vitamin B_{12} levels with recurrent aphthous stomatitis (RAS).

Patient and Method

This prospective cross sectional case control clinical study was on patients with recurrent aphthous stomatitis from October 2016 to April 2017. The study was approved by the Research Ethical Committee of the KBMS, and the subjects were seen in Khanzad Teaching Center in Erbil City.

Study groups. The RAS group was consisted of (50) consecutive patients (26 male, and 24 female mean age 35.6 ± 13 range 8-67 years) with a clinical diagnosis of RAS.

Inclusion criteria. RAS that recurred at least three times a year for at least one year [10]. **Exclusion criteria.** Patients taking any drug therapies or products containing iron or vitamins, patients with Behcet's disease, patients with known hematological diseases, patients with gastrointestinal diseases (coeliac disease, crohn's diseases), patients with renal failure, and patients under chemo or radiotherapy.

The control group. The control group was composed of (50) subjects (22 males and 28 female mean age 36.6±10) range 17 -60 years) clinically healthy subject living in the same geographical area as the RAS group, were consecutively enrolled as controls, attending Khanzad Teaching Center seeking dental treatment.

Inclusion criteria. Healthy age –and sex matched with RAS patients

Exclusion criteria. Clinically healthy with no clinical evidence and /or clinical history of recurrent aphthous or any oral lesions potentially associated with hematinic deficiency (oral candidiasis ,glossitis)

Methods. After obtained informed written consent, all patients completed a questionnaire for the assessment of age, gender, smoking habits, family history of recurrent aphthous, systemic disorders, and drug history. The family history was considered positive when recurrent aphthous was present in at least one first-degree relative (mother, father, sister, and brother), while patients who smoked more than one cigarette on a regular daily basis were accepted as active smokers.[36]After compiling the questionnaire, all patients underwent a careful extra and intra-oral examination and the diagnosis of RAS was based on the case history and findings of the clinical examination and those with recurrent aphthosis were classified as having minor, major or herpetiform aphthous ulcers, according to Stanley. [36]

The hemoglobin level, serum ferritin, folic acid, and vitamin B_{12} were investigated. The Central Public Health Laboratory did laboratory investigations. When we detect deficiency we gave the patient treatment by supplementation.

The normal laboratory ranges according references in the Central Public Health Laboratory as follow, female Hb=11.5-16.5g/dl, male Hb=13-18 g/dl, male ferritin =30-400ng/ml, female ferritin=13-150ng/ml, folic acid =3.7-17.5ng/ml, vitamin B 12=191-663 pg/ml as standard references.

Statistical analysis. Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 22). Chi square test of association was used to compare between proportions, and to show the association between the development of aphthous ulcer and some risk factors. Factors found to have significant association with the development of recurrent aphthous ulcer were entered into Binary logistic regression model, where the dependent variable was aphthous ulcer. A p value of $\leq$ 0.05 was considered statistically significant.

Results

Patient demography. The total RAS group size comprised of 50 patients, 26 males (52%) and 24 females (48%). The control group comprised of 50 subjects 22 males (44%) and 28 female (56%). No statistically significant difference between RAS group and control group regarding gender distribution as shown in table 1.

In RAS group 52% of them were male and 48%were female. There was no statistically significant difference regarding the gender (p value =0.54). Regarding the age group distribution, the RAS was more common in those between the 20-29 years.

The association between RAS and most common predisposing factors (family history, smoking, anemia and probable deficiencies of serum ferritin, folic acid and vitamin B_{12}.

The family history of recurrent aphthous was found to be significantly associated with recurrent aphthous, so most striking result was influenced by family history on RAS. Since it is 28% among RAS patients compared 9% among control subjects (p=0.001).

As far as smoking was considered, only 6% were smokers in the RAS group compared with 24% were smokers in control group, which was statistically significant (p=0.01) as shown in table 2.

Regarding anemia as shown in table 2, the level of Hb was low in 30% in RAS and 6%, in control group. Again it was statistically significant (p=0.02).

In this study we noticed that 62% of RAS patients were deficient in vitamin B_{12} whereas 24% in the control group had deficiency of vitamin B_{12} which was statistically significant (P=0.04). On the other hand, we noticed that only 8% of RAS group patients had low serum folic acid level compared with 16% in control group

Table 1: Gender and age distribution between study groups.

	RAS group No %	Control group No %	Total No %	P
Gender				
Male	26 (52)	22(44)	48(48)	0.42
Female	24 (48)	28(56)	52(52)	
Age(years)				
<20	2 (4)	2 (4)	4 (4)	
20-29	17 (34)	12 (24)	29 (29)	
30-39	15 (30)	15 (30)	30 (30)	
40-49	7 (14)	15 (30)	22 (22)	0.35
³50	9 (18)	6 (12)	15 (15)	
Total	50(100)	50(100)	100 (100)	

Table 2. The association between the development of aphthous ulcer and some risk factors.

	RAS Group No %	Control group No %	Total No%	P ≤ 0.05
Family history				
Yes	14 (28)	2 (4)	16(16)	**0.001**
No	36 (72)	48 (96)	84 (84)	
Smoking				
Yes	3 (6)	12 (24)	15 (15)	**0.01**
No	47 (94)	38 (76)	85 (85)	
Hb				
Low	15(30)	3(6)	8(18)	**0.02**
Normal or high	35(70)	47(94)	82(82)	
S.B_{12}				
Low	31 (62)	21 (42)	52 (52)	**0.04**
Normal or high	19 (38)	29(58)	48(48)	
S .folic acid				
Low	4(8)	8(16)	12(12)	0.21
Normal or high	46(92)	42(84)	88(88)	
S. ferritin				
Low	6(12)	8(16)	14(14)	0.46
Normal or high	44(88)	42(84)	86(86)	
Total	50 (100%)	50(100%)	100(100%)	

which is not statistically significant difference (P=0.2). Finally, the serum ferritin was below normal value in 12% of RAS group compared with 16% in control group which, is not statistically significant difference (P=0.4).

As shown in table 3 in the presence of family history is significantly associated with the development of RAS, family history is a significant risk for of RAS OR=8.4 (95% CI: 1.6-4.2).

The binary logistic regression analysis (Table 3) shows that smokers affected less than non smokers OR= 0.1 (95% CI: 0.04-0.9), proportion of the anemic was significantly associated with RAS occurrence OR=4.1 (95%CI: 1-16), so the positive family history, non smokers, and anemic were founded to have greater risk for having RAS.

Binary logistic regression analysis shows B_{12} has no role in developing RAS OR=1.7 (95% CI 0.6 -4.2) in this study.

Table 3. SPSS output of binary logistic regression analysis between recurrent aphhtous ulcer as dependent variable with some variables.

Variables	B	P	OR	95% C.I .for OR	
				Lower	Upper
Low Hb (anemia)	1.433	.042	4.191	1.053	16.687
Low B_{12}	.540	.239	1.716	.698	4.222
Family History	2.138	.012	8.484	1.602	44.925
Smoking	-1.653	.038	.191	.040	.910
Constant	-.554	.104	.575		

Discussion

Quality of life of RAS patients is affected in that the recurrent and painful intraoral mucosal lesions and increased salivation cause discomfort during eating, drinking, and speaking. Morbidity is quite high in RAS, because the exact etiology of RAS is still unknown; most patients with RAS are usually given some medications to relieve pain only, without doing investigations

This study revealed a higher number of male patients in RAS group but this is statistically no significant difference which is similar to the other study. [36] While other studies, were significantly more often in males. [37-39] In the study done by Scully female was more affected.[13] The high number of males in this study may be due to sample size.

The RAS can affect people of any age group. In this study about third of our patients (34%) is in the age group of (20-29) and mean of age is 35±13.6. Similarly, Complito *et al* in their study on adult patients reported that RAS was more common under the age of 38.5 years, there is no clear explanation why RAS was more frequent among this age group. No reference can give the explanation of this result. [27]

One of predisposing factors for recurrent aphthosis confirmed by our data was the role of genetic predisposition, demonstrated by the positive family history of recurrent aphthosis. and Koybasi *et al*[36] and Shohat -Zabarski et al [41] respectively, have reported that 42% and 54.2% of recurrent aphthosis patients had first-degree relatives with recurrent aphthosis. Another study done in Sulaimani in 2013 showed that 34.4% of patients reported had other family member suffered previously from RAS. [42] Compilato *et al* in 2010 [27] observed a strong association of positive family history for RAS in 53.1% of RAS group, and also Lopez *et al* in 2014 [30] founded association of family history with RAS. In agreement

with these results, this study showed a positive family history for recurrent aphthosis in 28 % of RAS group and this variable strongly correlated with recurrent aphthosis (OR = 8.4).

Whether this high degree of association is because of a direct genetic influence or similar social status, traditions, or habits of the family members is not known yet. So family history shows the strongest correlation.

Smoking is believed by some authors known to have a protective effect on RAS. This protective effect of smoking may be related to the increased keratinization of the oral mucosa in smokers. Keratin layer may possibly act as a mechanical and chemical barrier of the oral mucosa against minor traumas or microbial agents. **(35)** In this study the 6% (3patient) of RAS group were smoker compared with 24% (12 subjects) of control group (smoking were more common among control group than RAS group). This is in agreement with the study done by Koybasi *et al*[36] who found 20% were active smokers (7 patients) in the RAS group versus 43.7% (14 patients) in the control group (P = .052). This finding, statistically significant, and is concurrent with the literatures. [42,43] The data about the believed that tobacco has protective effect is limited and needs further studies. This doesn't mean that one should encourage smoking and, smoking is protective against aphthous.It is only an association need further clinical and epidemiological and pathological studies.

Anemia, ferritin, folate, and vitamin B12, play an important role in RAS, though the subject remains open to controversy. Compilato *et al* [27] reported a strong correlation of anemia to RAS. Routine hematological screening and tests for iron, folic acid, and vitamin B_{12} should be carried out in all patients with recurrent aphthosis to treat any deficiencies and prevent more important, related systemic manifestations. According to the result of this study, anemia was more frequent among RAS group patients (30%), and there was statistically significant difference between two groups (P=0.02). This is in agreement with study done by Compilato *et al* [27-] which showed that patients with recurrent aphthous were significantly anemic (34.4%) compared with (6.9%) controls, so Hb level patient can show association with RAS. Serum B_{12} status which is another proposed predisposing factor for RAS, and it has been shown that up to 20% of RAS patients may have at least one hemanitic deficiency. [22, 24] In this study, vitamin B_{12} deficiency was significantly more frequent in RAS (31%) than in control group (21%). With P value of 0.02

The question of how vitamin B_{12} deficiency causes RAS remained unclear, but the dramatic response to vitamin B_{12} replacement therapy and higher incidence of RAS in cases that have vitamin B_{12} deficiency suggest a direct role of this vitamin on the pathogenesis of RAS. [10, 22, 24] The most common etiology of vitamin B_{12} deficiency is food-cobalamin malabsorption resulting from gastric dysfunction. [43] Burgess and colleagues [44], in their study of 143 patients experiencing recurrent aphthous stomatitis, found that 26.6% of aphthous subjects demonstrated B_{12} deficiency in contrast to 12.6% of the controls. Piskin *et al* [10] found 35 patients with RAS have vitamin B_{12} levels significantly lower than 26 healthy controls, while significant differences were not found for the other assessed hematological factors. Volkov *et al* [45] used a randomized, double blind, placebo-controlled trial to confirm the use of vitamin B_{12} benefit in the treatment RAS. He suggested that daily 1000mcg vitamin B_{12} under the tongue may be preventive for aphthous ulcers after 5 and 6 months of use, despite the results of the above studies, the potential effect of vitamin B_{12} on aphthous ulcers is not well established, and the purpose of above study was to study whether the daily used vitamin B_{12} via oral supplements may be an effective strategy for reducing the number, duration, and pain of aphthous ulcers. Treatment with vitamin B_{12} by oral supplementation is safe, inexpensive and effective. This study could not confirm any significant differences, between the RAS subjects and controls for the serum folic acid and serum ferritin, which was in agreement with study done by Piskin *et al* [10] who found no statistical significant

difference between study groups, this is in disagreement with. Porter *et al* [29] study which have shown a significantly lower serum ferritin level (11.6%) in RAS patients compared with control group (4.9%).

Previous studies proposed possible etiologic roles of folic acid and minerals [10, 24]. However, Olsen [46] denied such relationship, which was in agreement with this study. The study done by Koybasi *et al* [36] founded only vitamin $B_{12}$to have significant correlation with RAS. This may be attributed to regional differences of our patients. Subjects in control group also have ferritin, and folic acid deficiencies. This may be due to dietary status of our population but we think that this issue should be studied further with a larger multicenter series.

The highest occurrence of RAS was found to be among positive family history, non smokers, anemic and vitamin B_{12} deficiency patients. Among these only anemia and vitamin B_{12}deficiency might be overcome by replacement therapy as we could not change the family history or encourage the patient to smoke, which is the same conclusion showed by many studies. [10, 27, 30, 37] The precise role of anemia, ferritin, vitamin B_{12}, or folic acid deficiency in the pathogenesis of recurrent aphthae is speculative, so further studies are therefore needed to clarify the importance of these parameters in RAS. It was not possible, by a clinical examination of the ulcers, to separate patients with an underlying hematological deficiency from those with no such abnormality. Taking all these findings into consideration, we can clearly state that RAS is a multifactorial disease, and we can conclude that positive family history, vitamin B_{12}deficiency, low Hb, and smoking status are among the studied possible etiologic factors.

Conclusions

Taking all these findings into consideration, we can clearly state that RAS is a multifactorial positive family history, non smoking, anemia, and vitamin B_{12} deficiency, are among the studied possible etiologic factors.

Recommendations

Further studies on large series should be able to clarify the importance and role of other vitamins and minerals in recurrent oral aphthae.

Routine haematological screening and tests for serum ferritin, folic acid and vitamin B_{12} should be done in all patients with recurrent aphthous to treat any identified nutritional deficiency and to prevent more important related systemic manifestations.

Conflict of interest

The authors reported no conflict of interests.

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