

Evaluation of Serum B12, Folic Acid, Iron, Ferritin, Total Iron Binding Capacity and Unsaturated Iron Binding Capacity in Patients with Recurrent Aphthous Stomatitis in Sulaimani City

Amir H.K. Kareem¹, Mohammed KM. Al-Emaam², Eisa Raad Jasim³

¹Specialist at Department of Dermatology, Shahid Tawfiq Hospital, Sulaymaniyah, Iraq, ²Specialist at Department of Dermatology, Al-Forat General Hospital Baghdad, Iraq, ³Specialist at Department of Internal Medicine, Al-Forat General Hospital Baghdad, Iraq

Abstract

Background: Many studies have demonstrated that iron, folate, Serum ferritin, Total iron binding capacity (TIBC), Unsaturated Iron Binding Capacity (UIBC), vitamin B1, B2, B6, B12 deficiencies occur in Recurrent aphthous stomatitis (RAS). The aim of this study was to evaluate serum level of vitamin B12, folate, ferritin, iron binding capacity, unsaturated iron binding capacity among patients with RAS.

Materials and Method

A cross sectional study carried out in Al-Sulaimani teaching center of dermatology for period from 1st of March to the end of June, 2014 on 30 RAS patients and 30 healthy controls. Data were collected were demographic information and results of haematinic investigations for selected patients. Data were analyzed using software SPSS program version 17.

Findings: Mean age of RAS patients was 40±14 years. Male and female patients were equally distributed. A significant difference was observed between patients and controls in family history of RAS (p<0.001). Mean B12 level of RAS patients was

190.6±80.3Pgm/ml, there was a significant difference in means of B12 between patients and controls (p=0.009). Mean serum folate level of the patients was 10.2±4.6 ngm/ml, there was a significant difference in means of serum folate between patients and controls (p=0.01). Meanserum iron level was 55.5±18.8 µg/dl, a significant difference was observed between patients and controls in means of serum iron (p<0.001). No significant difference was observed between patients and controls in means of ferritin, TIBC and UIBC (p>0.05).

Conclusions: Family history, serum B12 level, and serum iron are associated with RAS patients. It is important to investigate vitamin B12, folate and iron deficiencies in patients with RAS.

Keywords: *Aphthous, stomatitis, vitamins, anemia.*

Introduction

Recurrent aphthous stomatitis (RAS) is defined as the presence of recurring ulcers confined to the oral mucosa⁽¹⁾. RAS is characterized by recurrent, small, round, or ovoid ulcers often multiple with circumscribed margins, erythematous haloes and yellow or grey floors that present first in childhood or adolescence⁽²⁾. RAS occurs worldwide and are reported on every populated

Corresponding Author:

Amir H.K. Kareem

Department of dermatology, ShahidTawfiq Hospital,
Sulaymaniyah, Iraq

e-mail: amirkareem@yahoo.com

Mobile: +9647705330777

continent. RAS affects 20% of the population, with the incidence rising to more than 50% in certain groups of students in professional schools. Children from high socioeconomic groups may be affected more than those from low socioeconomic groups⁽³⁾.

The etiology of recurrent aphthous stomatitis is still not understood, although many predisposing and precipitating factors have been described: trauma, stress, changes in the immune system, sensitivity to certain types of food, or ingested substances such as preservative agents or the substances like cinnamaldehyde or sodium lauryl sulfate present in some toothpastes and iron, zinc, folate, vitamin B1, B2, B6, B12 deficiencies⁽⁴⁾.

The main pathogenetic event is the inflammatory response with production of inflammatory cytokines, prostaglandin E2, and nitric oxide. The pathophysiology of aphthous ulcers is not clearly understood. Alteration of local cell mediated immunity is often encountered in patients with recurrent aphthous ulcer. Systemic T- and B-cell responses have also been reported as altered in patients with RAU⁽⁵⁾. Three clinical presentations within recurrent aphthous ulcer has been identified which are : Aphthous ulcer minor (synonym Mikulicz ulcers) (MiAU); Aphthous ulcer major; and Herpetiform recurrent aphthous ulcer⁽⁶⁾.

In regards to differential diagnosis, RAS and recurrent intraoral herpes are the two most commonly occurring lesion in the mouth. However, there are several diseases that should be included in the differential diagnosis of patient who presents with a history of recurring ulcers of the mouth such as Behcet's syndrome, recurrent herpes simplex virus infection and recurrent erythema multiforme^(6, 7).

Materials and Method

Study design: A cross sectional study carried out in Al-Sulaimania teaching center of dermatology for a period from 1st of March to the end of June 2014. The target population was 30 cases all with the recurrent aphthous stomatitis attended the consultancy clinic of Al-Sulaimania teaching center of dermatology, with 30 controls without oral aphthous ulcer. Adult patients who consented to participate in this study were recruited. Exclusion criteria included 1) Systemic conditions, and other diseases having oral ulceration likes Reiter's syndrome, Behcet's syndrome, Crohn's disease and gastrointestinal problems, Sweet's syndrome (acute febrile neutrophilic dermatosis), recurrent herpes

simplex virus infection, recurrent erythema multiforme, PFAPA (periodic fever, Aphthae, pharyngitis and cervical adenitis) and HIV (aphthous like ulceration).²⁾ No consumption of folic acid, vitamin B12 and iron in drug form and other supplements during the past 6 months.

Data Collection: The collection of data was done through direct interview in consultancy clinic of Dermatology center with each patient. Each patient was diagnosed previously by a dermatologist as RAS. The researcher interviewed the controls and filled a prepared questionnaire. The questionnaire included the followings: a) History: age, gender, family history, durations, times and number of recurrence, and number of ulcers. b) Symptoms: such as pain, difficulty in eating and drinking. Drugs history: topical, systemic, or mixed. c) Examination: oral cavity is examined using torch light, all the parts of oral cavity are examined, recording numbers and type of ulcers.

Investigations: The hematological tests were carried out in central laboratory of Al-Sulaimania teaching hospital, laboratory of SHAHID HADI outpatient's clinic, after taking blood samples (5ml of blood) for both patients and controls. a) Serum B12 tests result: was done Enzyme Immunoassay with AIA-Pack B12 Kits[®]. Normal value in pg/mL (173-700)⁽⁸⁾. b) Serum ferritin test result: was done by Immuno-Enzymometric Assay with ST AIA-Pack FER[®]. Normal values of serum ferritin in ng/ml are 30-350 for male and 20-250 for female⁽⁹⁾. c) Serum iron: was done by direct method using BIOLABO reagents (Ferene)[®]. Normal values of serum iron in µg/dl are (55-145)⁽¹⁰⁾. d) Serum folate: was done by Bio-Rad Laboratories' "Quantaphase II Folate/Vitamin B12" Radio assay Kit[®]. Normal values in ng/ml (3-17)⁽¹¹⁾. e) TIBC and UIBC: was done by Treatment of Specimens for the Determination of Total Iron Binding Capacity and unsaturated iron binding capacity of Transferrin in human serum and plasma using BIOLABO Reagents[®]. Normal values of TIBC (250-400 µg/dl)⁽¹²⁾. Normal values of UIBC (180-280 µg/dl)⁽¹³⁾.

Statistical analysis: All patients' data entered using Statistical Package for Social Sciences (SPSS) version 17. Descriptive statistics presented as (mean ± standard deviation) and frequencies as percentages. Multiple contingency tables conducted and appropriate statistical tests performed, Chi-square used for categorical variables. In all statistical analysis, level of significance (p value) set at ≤0.05 and the result presented as tables

and/or graphs. Odds ratio (OR) was calculated using binary logistic regression to identify factors associated with RAS.

Findings: Mean age of RAS patients was 40±14 years, age group 30-39 years was the prevalent. Male and female gender was equally distributed, male: female ratio was 1. Positive family history represented 53.3% of RAS patients. Painful ulcer was present among 27 (90%) RAS patients. Seven (23.3%) patients had single ulcer and 23 (76.7%) had multiple ulcers. The most prevalent frequency of ulcer recurrences was 4 times/year, 19 (63.3%) patients had 4 times/year recurrences of ulcer. Mean aphthous duration was 8.2±3.5 years, 50% of the patients had aphthous duration > 9years.

Mean age of controls was 36±11 years with prevalent age group 30-38 years. Males were 16 and females were 14. Controls with family history of RAS were only two. The means of B12, serum folate, serum ferritin, TIBC, UIBC and serum iron of the controls were 240±60.9 Pgm/ml, 13.1±4.7 ngm/ml, 55.1±54.6 ngm/ml, 333.9±78.8 µg/dl, 300.1±38.0 µg/dl and 78.8±42.2 µg/dl, respectively.

There was no significant difference between patients and controls regarding age groups and gender of them (p>0.05). A significant difference was observed between patients and controls in family history of RAS (p<0.001). The odds of having RAS among patients with positive family history were 16 times more than those with negative family history, table 1.

Table 1: Distribution of patients and controls according to demographic variables (no=60).

Variable	Patients		Control		χ^2	P	OR
	No.	%	No.	%			
Age							
<20 years	2	6.7	1	3.3	3.6*	0.4	-
20-29 years	4	13.3	6	20.0			
30-39 years	9	30.0	10	33.3			
40-49 years	7	23.3	10	33.3			
≥ 50 years	8	26.7	3	10.0			
Gender							
Male	15	50.0	16	53.3	0.06	0.7	0.8 CI[0.3-2.4]
Female	15	50.0	14	46.7			
Family history							
Positive	16	53.3	2	6.7	15.5	<0.001	16 CI[3.2-79.5]
Negative	14	46.7	28	93.3			

*Fishers Exact test.

Mean B12 level of RAS patients was 190.6±80.3 Pgm/ml, 12 (40%) patients had low vitamin B12 level. Mean serum folate level of the patients was 10.2±4.6 ngm/ml, two (6.7%) patients had low serum folate level. Mean ferritin level of the patients was 43.3±47.6 ngm/ml, 50% of the patients had low ferritin level. Mean TIBC of the patients was 338.9±58.0 µg/dl, 3 (10%) patients had low TIBC. Mean UIBC of the patients was 300.1±38.0 µg/dl, 3 (10%) patients had low UIBC. Mean serum iron level was 55.5±18.8 µg/dl, 12 (40%) patients had low serum

iron. All these findings were shown in table 4. Table 4: Hematological parameters of the patients (no=30).

There was a significant difference in means of B12 between patients and controls (p=0.009). A significant low serum B12 level was observed among RAS patients. There was a significant difference in means of serum folate between patients and controls (p=0.01). A significant low serum folate level of the patients was observed. A significant difference was observed between

patients and controls in means of serum iron (p<0.001). The mean of serum iron was significantly lower among patients. No significant difference was observed between

patients and controls in means of ferritin, TIBC and UIBC (p>0.05), table 7 and figure 4.

Table 2: Distribution of patients and controls according to means of hematological parameters (no=60).

Variable	Patients	Control	t	P
	Mean±SD	Mean±SD		
Serum B12	190.5±80.3	240±60.8	2.6	0.009
Serum folate	10.2±4.5	13.1±4.6	2.4	0.01
Serum ferritin	43.2±47.5	55.1±54.5	0.8	0.3
TIBC	338.8±57.9	333.9±78.7	0.2	0.7
UIBC	300±38	294±33.5	0.6	0.5
Serum Iron	55.4±18.8	87.7±42.2	3.8	<0.001

Binary logistic regression analysis revealed that family history of RAS (p=0.002), serum B12 (p=0.003) and serum iron (p=0.008) were significant predictors of recurrent aphthous stomatitis, table 8.

Table 3: Results of binary logistic regression

Variable	β	SE	Wald χ ²	P	OR
Constant	6.7	2.5	7.05	0.008	0.001
Family history	4.9	1.5	9.6	0.002	0.007
Serum B12	0.02	0.009	9.05	0.003	1.02
Serum Folate	0.18	0.09	3.5	0.058	0.8
Serum Iron	0.07	0.02	7.07	0.008	1.07

Discussion

RAs is a common presentation for both dermatology and general practice clinics. Considerable proportion of people are unaware of the method to reduce its prevalence. The demographic characteristics of our sample are consistent with results Fariba I, et al study in Iran (2005) that reported equal male to female ratio and mean age of patient was 30.5 years⁽¹⁴⁾. Our findings are also close to results of Tarakji B, et al study in Saudi Arabia (2012)⁽¹⁵⁾ that reported prevalent age group of AS patients 30-39 years but males were more than females, on other hand, these findings are inconsistent with Abdullah MJ, et al study in Al-Sulimaniya (2013)⁽¹⁶⁾ that reported main age group for RAS was 20-29 years and females more than females.

The prevalence of RAS among adults was significantly higher than that (17.9%) reported in US adult population aged over 17 years⁽¹⁷⁾. More than half of

the RAS patients had positive family history. Reports of family history were frequent in this population (53.3%), as observed in the previous literatures^(18, 19) supporting a genetic involvement in the etiology of RAS.

The present study revealed a significant decrease in mean of serum B12 among RAS patients (p=0.009). Mean of serum B12 for patients was 190.5±80.3 Pgm/ml and that for controls was 240±60.8 Pgm/ml. Volkov I, et al study in USA (2009)⁽²⁰⁾ concluded that vitamin B12 treatment, which is simple, inexpensive, and low-risk, seems to be effective for patients suffering from RAS, regardless of the serum vitamin B12 level.

Burgan SZ, et al study in Jordan (2005)⁽²¹⁾ reported that Patients with recurrent aphthous stomatitis have more hematinic deficiencies, particularly vitamin B12 deficiency, compared with controls. Correction of these hematinic deficiencies could help in the management of the disease. Liu HL, et al study in Southern Korea

(2013)⁽²²⁾ resulted vitamin B12 therapy to have a statistically significant benefit on pain relief among recurrent aphthous stomatitis sufferers.

Binary logistic regression analysis revealed that the main risk factors for RAS in this study were family history ($p=0.002$), serum B12 ($p=0.003$) and serum iron ($p=0.008$). A genetic predisposition for the development of aphthous ulcer is strongly suggested as about 40% of patients have a family history and these individuals develop ulcers earlier and are of more severe nature. Various associations with HLA antigens and RAS have been reported. These associations vary with specific racial and ethnic origins⁽¹⁸⁾.

It has long been thought that iron, folate and vitamin B12 deficiencies play an important role in RAS, but some controversy does exist. Wray D, et al⁽²³⁾ reported that hematinic deficiencies affect up to 21% of adult patients with RAS, and when they replaced the deficient element, 59% of the patients showed resolution of RAS and 28% showed significant improvement. However, other studies have shown that patients with RAS and controls had comparable serum iron, folate or vitamin B12^(24, 25).

Conclusion

In conclusion, family history, serum B12 level, and serum iron are associated with RAS patients. It is important to investigate vitamin B12, folate and iron deficiencies in patients with RAS.

Conflict of Interest: The authors have no conflict of interest

Source of Funding: Not available

Ethical Clearance: Ethical approval was obtained from Al- Sulaimania teaching hospital

References

- Chavan M, Jain H, Diwan N, Khedkar S, Shete A, Durkar S. Recurrent aphthous stomatitis: a review. *Journal of oral pathology & medicine*. 2012;41(8):577-83.
- Jurge S, Kuffer R, Scully C, Porter S. Mucosal disease series. Number VI Recurrent aphthous stomatitis *Oral Dis*. 2006;12(1):1-21.
- Natah S, Konttinen YT, Enattah NS, Ashammakhi N, Sharkey K, Häyrinen-Immonen R. Recurrent aphthous ulcers today: a review of the growing knowledge. *International journal of oral and maxillofacial surgery*. 2004;33(3):221-34.
- Baccaglini L, Lalla RV, Bruce AJ, Sartori V, Valinotti JC, Latortue MC, Carrozzo M, et al. Urban legends: recurrent aphthous stomatitis. *Oral diseases*. 2011;17(8):755-70.
- Glen H. *The Dermatology Lexicon Project (DLP)*. 2005.
- Boras VV, Savage N. Recurrent aphthous ulcerative disease: presentation and management. *Australian dental journal*. 2007;52(1):10-5.
- Scully C, Porter S. Oral mucosal disease: recurrent aphthous stomatitis. *British Journal of Oral and Maxillofacial Surgery*. 2008;46(3):198-206.
- Care R. *The NHS Atlas of Variation in Diagnostic Services. Reducing Unwarranted Variation to Increase Value and Improve Quality*. 2013.
- Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: past, present and future. *Biochimica et Biophysica Acta (BBA)-General Subjects*. 2010;1800(8):760-9.
- Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J. *Harrison's principles of internal medicine*. 2001.
- Coppen A, Bolander-Gouaille C. Treatment of depression: time to consider folic acid and vitamin B12. *Journal of psychopharmacology*. 2005;19(1):59-65.
- Dwyer JT. Dietary standards and guidelines: similarities and differences among countries. *Present knowledge in nutrition*. 2012;1110-34.
- Dimeski G. Interference testing. *The Clinical Biochemist Reviews*. 2008;29(Suppl 1):S43.
- Fariba I, Taghi G, Mazdak E, Maryam T, Hossein S, Shahla E, et al. The efficacy of 3% diclofenac in 2.5% hyaluronan gel base for treatment of recurrent aphthous stomatitis (RAS): A double blind study. *Egyptian Dermatology Online Journal*. 2005;1(2):4.
- Tarakji B, Baroudi K, Kharma Y. The effect of dietary habits on the development of the recurrent aphthous stomatitis. *Nigerian medical journal: journal of the Nigeria Medical Association*. 2012;53(1):9.
- Abdullah MJ. Prevalence of recurrent aphthous ulceration experience in patients attending Piramird dental speciality in Sulaimani City. *Journal of clinical and experimental dentistry*. 2013;5(2):e89.
- Sawair FA. Does smoking really protect from

- recurrent aphthous stomatitis? Therapeutics and clinical risk management. 2010;6:573.
18. McCullough MJ, Abdel-Hafeth S, Scully C. Recurrent aphthous stomatitis revisited; clinical features, associations, and new association with infant feeding practices? *Journal of oral pathology & medicine*. 2007;36(10):615-20.
 19. Compilato D, Carroccio A, Calvino F, Di Fede G, Campisi G. Haematological deficiencies in patients with recurrent aphthosis. *Journal of the European Academy of Dermatology and Venereology*. 2010;24(6):667-73.
 20. Volkov I, Rudoy I, Freud T, Sardal G, Naimer S, Peleg R, et al. Effectiveness of vitamin B12 in treating recurrent aphthous stomatitis: a randomized, double-blind, placebo-controlled trial. *The Journal of the American Board of Family Medicine*. 2009;22(1):9-16.
 21. Burgan SZ, Sawair FA, Amarin ZO. Hematologic status in patients with recurrent aphthous stomatitis in Jordan. *Saudi medical journal*. 2006;27(3):381.
 22. Liu H-L, Chiu S, Chen K-H. Effectiveness of Vitamin B12 on recurrent aphthous stomatitis in long term care: a systematic review. *JBIC Database of Systematic Reviews and Implementation Reports*. 2013;11(2):281-307.
 23. Wray D, Ferguson M, Hutcheon A, Dagg J. Nutritional deficiencies in recurrent aphthae. *Journal of oral pathology & medicine*. 1978;7(6):418-23.
 24. Piskin S, Sayan C, Durukan N, Senol M. Serum iron, ferritin, folic acid, and vitamin B12 levels in recurrent aphthous stomatitis. *Journal of the European Academy of Dermatology and Venereology*. 2002;16(1):66-7.
 25. Ship JA, Chavez EM, Doerr PA, Henson BS, Sarmadi M. Recurrent aphthous stomatitis. *Quintessence in international*. 2000;31(2).