



Serum and Salivary Ferritin Level in Relation to Iron Deficiency Patient with Recurrent Aphthous Stomatitis

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Abstract

Background: Recurrent aphthous stomatitis (RAS) is one of the most widespread disorders affecting the oral mucosa, with a prevalence of 50–66%. This study investigated the serum and salivary levels of ferritin between participants with recurrent aphthous ulcers and healthy. Methods: A total of 75 individuals participated in the research separated into three groups, clinically healthy control group (n=25), iron deficiency anemic patient with recurrent aphthous stomatitis (n=25), and iron deficiency anemic patient without recurrent aphthous stomatitis (n=25). The enzyme-linked immunosorbent assay measured the serum and salivary ferritin levels. Result: the serum and salivary levels of ferritin were significantly lower ($p<0.05$) in anemic groups compared to healthy controls. In the salivary ferritin, there was a statistically significant difference between anemic patients without aphthous ulcer and anemic with aphthous ulcer ($p=0.015$), while in the serum ferritin, there was non-significant difference. Conclusion: The severity of ferritin deficiency plays as a cofactor for the development and recurrence of recurrent aphthous stomatitis.

Introduction:

Persistent oral ulcers without other disease symptoms are the hallmark of the common oral mucosa condition known as recurrent aphthous stomatitis (RAS) ^(1,2).

Patient's quality of life is decreased by ulcers, which are painful and make it difficult to swallow, talk, or eat ^(3,4). Although a 5-25% prevalence has been

observed, aphthous stomatitis prevalence mostly depends on the study population ⁽⁵⁾. According to studies, oral aphthous is more prevalent in adult women, those under 40, whites, nonsmokers, and those with a higher socioeconomic status ⁽⁶⁾.

There are three known forms of RAS: minor, major, and herpetiform types ⁽⁷⁾. 80% of RAS patients have the most common kind, minor RAS. Minor RAS oral ulcerative lesions range in diameter from 3 to 10 mm. The buccal and labial mucosae are frequently impacted and typically develop from nonkeratinized oral mucosae. An erythematous macule with prodromal burning or stinging sensations for a few hours to one or two days may arise before the lesion. The oral ulceration then develops, followed by the appearance of a yellow-white fibrinopurulent pseudo membrane over it. In 7–14 days, oral ulcers recover without leaving scars. RAS is a member of the category of ulcerative, inflammatory, and chronic oral mucosa disorders. Although the etiopathogenesis of this illness is yet unknown, it is thought to be multifactorial ⁽⁸⁾. Previous research has found that genetically mediated changes significantly influence the development of the disease in innate and acquired immunity. Genetic predisposition, viral and bacterial infections, food allergies, vitamin and microelement deficits, systemic disorders, hormonal imbalance, mechanical injuries, and stress are factors that alter the immune responses in RAS ⁽⁹⁾.

A failure in hemoglobin synthesis causes red blood cells to be unusually small (microcytic) and have lower hemoglobin levels (hypochromic), which is the hallmark of iron deficiency anemia. As a result, the blood's ability to provide oxygen to body tissues and cells is diminished ⁽¹⁰⁾. The effects of iron deficiency anemia on motor and mental development in children and adolescents, as well as impaired work capacity in adults, visual and auditory functioning, and poor cognitive development in children, are all possible. There is some proof that iron deficiency without anemia impacts adolescent girls' cognitive and makes adult women tired ⁽¹¹⁾. Ferritin, which is the body's form of iron storage, is

present. An intracellular protein called ferritin is made up of 24 subunits that surround an iron core that may include 4,000–4,500 iron atoms ⁽¹²⁾. Serum Ferritin (SF) was identified in the 1930s, and a clinical test was developed in the 1970s ⁽¹³⁾. Ferritin is the main intracellular iron-storage protein that maintains iron in a non-toxic and soluble form; small amounts of ferritin are secreted into the body's plasma. In the absence of inflammation, there is a positive correlation between the plasma (or serum) ferritin concentration and total body iron storage.

Low serum ferritin values are an indication of depleted iron levels. Normal serum ferritin concentrations vary with age and gender; at birth, concentrations are high, rise during the first two months of life, and then decline during later infancy; concentrations begin to rise again at approximately one year of age and continue to grow into adulthood ^(14,15). Females have lower values than males beginning in adolescence. Agarwal and coworkers reported ferritin in saliva in 1984 ^(14,16). According to research by Agarwal and coworkers, salivary ferritin levels were found to be significantly higher than normal, who also noted alterations in ferritin levels associated with iron deficient anemia ⁽¹³⁾. Nonetheless, salivary ferritin and iron may be considered as novel discriminating indices with the benefit of a simple method ⁽¹⁷⁾.

Methods

The study was approved by the institutional ethics committee issued by the university of Baghdad college of dentistry (Ref. number 750/750722 on 28-12-2022). Samples size was calculated by Using G power 3.1.9.7 (Universitat Kiel, Germany) with the power of study=85%, alpha error of probability=0.05 two-sided, assuming effect size of F is 0.4 (large effect size), with 3 groups, the sample size was estimated to be 75 subjects (25 subjects for each group).

The samples were collected at Alsader medical center in Najaf city and Alfurat hospital in Alkufa / Najaf city from February 2022 to the end of August 2022.

The international center for research and development (ICRD) in Baghdad did laboratory work, and the operator was blinding about all groups.

Subjects were divided into three groups:

Control group (n=25)

Experimental groups: iron deficiency anemia without aphthous stomatitis (n=25) and iron deficiency anemia with aphthous stomatitis (n=25).

Diagnosis requires the presence of round, yellow-white ulcers that are symmetrical and surrounded by a halo of erythema and a detachable membrane that heals with no scarring. Chronic smokers and drinkers were excluded, as were those with a history of Behcet's illness, hypertension, cardiac diseases, hepatic, renal, and hematological disorders, as well as those taking methotrexate, nonsteroidal anti-inflammatory medicines, sulphonamides, rifampin, and vancomycin⁽¹⁸⁾. The hematologist diagnosed iron deficiency anemia (IDA) in the blood film.

The subjects in the control group had no history of illness or RAS lesions at the time of data collection. Demographic information from patients, including their gender and age, for all patients and controls participant, 5ml of venous blood was obtained from each individual⁽¹⁹⁾. Saliva was collected for all participants into plastic containers (CNWTC, Jiangsu, China) between 9 and 11 am under the same conditions. The subjects were instructed to rinse and wash their mouths with distilled water before collecting saliva. Saliva was collected before or at least one hour after a meal. Unstimulated saliva was collected by draining by asking the participant to sit with their head bent with an open mouth. Samples (serum and saliva) were centrifuged (Rotofix 32A, Germany) at 3000 rpm for 10 minutes, and the clear supernatants were stored in Eppendorf tubes (CNWTC/OEM, Jiangsu, China) inside a deep freezer at (-80) C (Angelantoni Life Science, Massa Martana PG, Italy) until further analysis^(20,21).

Biochemical analysis

Human Specific Enzyme-Linked Immunosorbent Assay (ELISA) kits from (MyBioSource, San Diego, California, USA) were utilized to determine salivary and serum ferritin levels per the manufacturer's instructions. Salivary samples were thawed and labeled before the procedure. Kits' sensitivity values were 0.10 ng/mL, with a detection range of 0.16-10 ng/mL. The optical density was measured at 450 nm. The operator who performed the procedures was blinded to the tested groups.

Statistical analysis

Shapiro-Wilk test was used for the assessment of normality using SPSS version 28 (Statistical Package for Social Science), and all data revealed normal distribution ($p > 0.05$). Data analyses were performed by chi-square (χ^2), one-way analysis of variance (ANOVA), and Games-Howell for multiple groups comparison. We defined statistical significance as a P-value less than 0.05.

Results

The current study includes 25 patients with IDA and RAS, 25 patients without IDA and RAS, and 25 control group. Participants ranged in age from 20 to 49, with 51 females (68% of total) and 24 males (32%) (Table 1).

Laboratory findings

Salivary ferritin

Results showed a statistically significant difference ($P < 0.05$) in the mean of ferritin in the study groups compared to the control group. Table(2). using the Games-Howell test revealed that there was a statistically significant difference in anemic patients without aphthous ulcer compared to the control group ($P < 0.05$), there was a statistically significant difference in anemic patients with aphthous ulcer compared to the control group ($P < 0.05$), and there was a statistically significant difference between patients. Anemic without aphthous ulcer and anemic with aphthous ulcer groups ($P > 0.05$).

Serum ferritin

Results showed a statistically significant difference ($P < 0.05$) in the mean of ferritin in the study groups compared to the control group Table(2). Using the Games-Howell test revealed a statistically significant difference in anemic patients without aphthous ulcer compared to the control group ($P < 0.05$), there was a statistically significant difference in anemic patients with aphthous ulcer compared to the control group ($P < 0.05$). At the same time, there was a statistically non-significant difference between anemic patients without aphthous ulcer and anemic with aphthous ulcer ($P > 0.05$).

Discussion:

The purpose of this investigation was to evaluate the serum and salivary ferritin levels in patients with recurrent aphthous ulcers. Individuals suffering from recurring aphthous ulcers experience considerable discomfort. RAS can have a significant influence on a patient's quality of life since it causes an increase in saliva flow, impedes daily tasks such as eating, drinking, and speaking, and increases saliva production. As the underlying etiology of RAS is unknown, the primary objective of treatment is pain reduction⁽²²⁾. In the present investigation, ferritin levels in serum and saliva were examined between IDA patients with RAS, IDA patients without RAS, and healthy controls. There was no statistically significant difference in the demographic features of the experimental and control groups. The fact that women have a high propensity to develop anemia may contribute to explaining the greater frequency of RAS in females, as demonstrated by a prior study⁽²³⁾. It has been believed for a long time that deficiencies in iron, folate, and vitamin B12 have an important impact on RAS^(24,25). However, there is some controversy about this theory. Al-Alimi⁽²⁴⁾ found that up to twenty-one percent of adult patients with RAS had hematinic impairments.

When the missing element was supplied, fifty-nine percent of patients achieved RAS remission, and twenty-eight percent saw considerable improvement. However, other research showed that the serum levels of iron, folate, and vitamin B12 were comparable between RAS patients and controls⁽²⁶⁻²⁸⁾. There was a statistically significant difference in the ferritin mean between the experimental and control groups in the current study.

The study finds that although the mean of IDA with RAS was lower than IDA without RAS, there was no statistically significant distinction between them. The growth of epithelial cells depends on the iron-dependent enzyme. Epithelial cells turn over more quickly with iron deficiency, resulting in immature or atrophic mucosa⁽²⁹⁾. This weak mucosa is more delicate and susceptible to pathophysiological conditions such as ulcer formation⁽³⁰⁾. Moreover, reduced oxygen levels in anemic patients also translate to lower oxygen levels in other tissues, including the oropharynx's mucosal epithelium. Atrophic mucosa is caused by the collapse of cells, which is aggravated by inadequate oxygen saturation. Those with anemia may be more susceptible to RAS development due to atrophic oral epithelium⁽³¹⁾.

Conclusion

Routinely measurement of ferritin and hematological state of the patient with recurrent aphthous stomatitis to exclude their cause of ulcers. Ferritin, B 12, folic acid, and hematological deficiencies should be replaced to reduce RAS's severity and recurrence.

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Conflicts of Interest Statement: No conflicts of interest

Table (1): Age and gender distribution of participants with comparisons significant

		IDA without RAS		IDA with RAS		Control		P value
		No	%	No	%	No	%	
Age (years)	<20years	-	-	4	16.0	2	8.0	0.083
	20---29	6	24.0	3	12.0	9	36.0	
	30---39	4	16.0	5	20.0	3	12.0	
	40---49	12	48.0	7	28.0	5	20.0	
	50---59	3	12.0	1	4.0	3	12.0	
	=>60years	-	-	5	20.0	3	12.0	
	Mean±SD (Range)	38.7±9.8 (22-56)		39.8±15.2 (16-65)		37.6±14.0 (18-64)		0.847
Gender	Male	6	24.0	9	36.0	9	36.0	0.576
	Female	19	76.0	16	64.0	16	64.0	

significant P<0.05

No significant P>0.05

Table (2): Mean and SD of vitamin B12

		Anemic without Aphthous ulcer	Anemic with Aphthous ulcer	Control	P value
Saliva Ferritin (ng/mL)	Mean±SD (Range)	13.548±1.519 (11.479-16.46)	12.285±1.557 (9.384-14.247)	24.804±4.396 (21.8-37.898)	0.0001
Serum Ferritin (ng/mL)	Mean±SD (Range)	19.144±1.599 (15.558-21.82)	18.438±1.508 (15.811-20.82)	41.271±7.556 (28.22-49.95)	0.0001

significant P<0.05

No significant P>0.05

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