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Hematological and Biochemical Alterations in Patients with Recurrent Aphthous Stomatitis

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ABSTRACT

Recurrent aphthous stomatitis (RAS) is a common oral mucosal disorder characterized by painful, recurring ulcers. Its etiology remains multifactorial, with potential links to hematological and biochemical imbalances. **Objectives:** To compare the hematological and biochemical profiles of adult patients with and without recurrent aphthous stomatitis (RAS) attending the outpatient department of a teaching hospital in Hyderabad, Pakistan. **Methods:** A total of 130 participants were enrolled using a non-probability convenience sampling method, with 65 individuals in the RAS group and 65 healthy controls. Standardized clinical oral examinations were conducted to evaluate the presence, number, and location of ulcers in RAS patients, while controls were examined to confirm the absence of oral mucosal lesions. Under sterile conditions, 6 mL of blood was collected from each participant for hematological and biochemical analyses, including serum levels of cortisol, ferritin, iron, folic acid, and vitamin B12, measured using an auto-analyzer. **Results:** Independent t-tests revealed significantly higher cortisol levels in RAS patients (459.2 ± 49.6 nmol/L) compared to controls (232.4 ± 119.4nmol/L, p=0.001). Conversely, ferritin, iron, and folic acid levels were significantly lower in the RAS group (p=0.001). Hemoglobin (12.9 ± 2.1g/dL vs 14.0 ± 1.4g/dL, p=0.001) and hematocrit (37.88 ± 8.14% vs 40.85 ± 5.81%, p=0.018) were also significantly lower in RAS patients, while differences in RBC, WBC, and platelet counts were not statistically significant. **Conclusions:** These findings suggest potential connections between RAS and altered specific biochemical and hematological markers, including cortisol, ferritin, iron, folic acid, hemoglobin, and hematocrit.

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is one of the most common inflammatory disorders of the oral mucosa, characterized by the repeated formation of painful, round or oval ulcers. It affects approximately 5–25% of the general population, with regional variation depending on age, diet, and socioeconomic conditions [1, 2]. While minor RAS accounts for nearly 80% of cases, typically presenting as shallow ulcers (<1 cm) on non-keratinized oral mucosa, major and herpetiform variants are more severe and often leave scars upon healing [3, 4]. Although RAS is clinically well-recognized, its exact etiology remains unclear and is likely multifactorial. Contributing factors reported in literature include genetic predisposition, local trauma, stress, food allergies, hormonal imbalances, immune dysregulation, and nutritional deficiencies [5–7]. In particular, iron, folate, and vitamin B12 deficiencies have been frequently implicated in individuals with RAS, given their roles in maintaining epithelial integrity and hematopoiesis [8]. Vitamin B12 deficiency, for instance, has been associated with epithelial atrophy, megaloblastic

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anemia, and oral mucosal changes, while folic acid is essential for DNA synthesis and repair of oral mucosa [9]. Iron deficiency, best assessed through serum ferritin, impairs epithelial regeneration and immune response, and is a recognized predisposing factor for oral ulcers [10, 11]. In addition to nutritional factors, psychological stress has also been linked to RAS in several studies, often assessed through serum cortisol levels. Elevated cortisol, a stress biomarker, may reflect underlying anxiety or emotional distress contributing to RAS recurrence. However, conflicting evidence exists, as some studies have failed to demonstrate a clear association between stress markers and RAS [12]. Despite extensive international research on recurrent aphthous stomatitis (RAS), there is limited context-specific data from South Asia, particularly Pakistan, examining the role of nutritional deficiencies, hematological profiles, and systemic inflammatory markers. Given regional dietary habits, healthcare access, and genetic variability, local investigation is crucial to identify relevant contributing factors and improve targeted management. Investigating hematological (e.g., anemia, leukocyte counts) and biochemical (e.g., vitamin B12, folate, iron) changes would provide insights into possible systemic causes, improve diagnostic accuracy, and guide more effective management strategies, ultimately enhancing patient outcomes.

This study aims to determine the hematological and biochemical changes among patients with and without RAS presented to the dental OPD at Liaquat University Hospital and the Diagnostic Research Laboratory, Hyderabad, Pakistan.

METHODS

This comparative cross-sectional study was done from April 2020 to May 2022 at the Dental Outpatient Department (OPD) of Liaquat University Hospital and the Diagnostic Research Laboratory, Hyderabad, Pakistan. The study was approved by the ERC committee of LUMHS vide letter no. LUMHS/REC/-818. Assuming a 10% prevalence of recurrent aphthous stomatitis [3], with a 5% margin of error and a 95% confidence interval for a target population of 20,000, an equal allocation was made, with 69 individuals in the RAS group and 69 in the healthy control group. Following recruitment, eight participants withdrew, resulting in a final sample size of 130, with 65 participants in each of the two groups. A non-probability convenience sampling technique was employed due to the difficulty of obtaining a random sample of individuals with RAS from the general population. The inclusion criteria for the RAS group required patients to have a history of recurrent aphthous ulcers for at least six months, with a minimum of two ulcer episodes within the past year. The control group comprised healthy individuals with no history of recurrent oral ulcers or any other oral mucosal disease. Patients with pre-diagnosed or clinically confirmed iron deficiency anemia, vitamin B12 or folate deficiency anemia were excluded at the time of recruitment to avoid confounding pre-existing systemic causes of oral ulceration. Participants with chronic systemic diseases (e.g., diabetes, autoimmune disorders), pregnancy or lactation, tobacco or betel nut use, smoking history, or use of medications affecting oral ulcers or lab parameters were also excluded. All individuals who met the respective inclusion criteria, provided with informed consent before their enrollment in the study. Data and clinical findings were recorded for all participants. All participants underwent a standardized clinical oral examination conducted by two experienced clinicians with more than five years of experience in a filter clinic under adequate lighting conditions. In the RAS group, the presence, number, and location of ulcers were recorded, along with associated symptoms such as pain and discomfort. Active ulcers, if present at the time of examination, were documented in detail, including their morphological characteristics. Additionally, participants were asked about ulcer episodes. For the control group, a thorough oral examination was performed to confirm the absence of any active or past signs of recurrent aphthous ulcers or other oral mucosal lesions. The overall oral health status, including mucosal integrity, was assessed to rule out any conditions that could mimic RAS. 6 ml of Blood samples were collected between 8:00–10:00 AM, within 24 hours of clinical diagnosis of active RAS or 2 weeks of a recent episode, confirmed through oral examination. Hematological parameters (CBC, RBC indices, WBC count, platelets) were analyzed using the Sysmex XN-1000 hematology analyzer. Serum cortisol, ferritin, iron, folic acid, and vitamin B12 levels were measured using Electrochemiluminescence Immunoassay (ECLIA) on a Cobas e 601 auto-analyzer (Roche Diagnostics). Data were analyzed using SPSS version 25.0. Quantitative variables (e.g., cortisol, ferritin, iron, folic acid, vitamin B12, hemoglobin, hematocrit, RBC, WBC, and platelet count) were expressed as mean ± SD and tested for normality using the Shapiro-Wilk test. Qualitative variables (e.g., gender, age group, residence, education) were presented as frequencies and percentages. The primary outcomes were differences in biochemical and hematological parameters between the RAS and control groups. Independent Sample t-tests were applied to compare means of normally distributed quantitative variables between groups. A p-value<0.05 was considered significant.

RESULTS

A total of 130 participants were enrolled, with equal numbers in the RAS and control groups. Male predominated in both groups (72.3% in RAS, 70.8% in controls). Most participants were aged 18–30 years, followed by 31–45

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years. Urban residents constituted the majority (63.1% in RAS, 60.0% in controls). Educational levels were comparable across groups, with roughly one-third having primary education or less, and around 30% being graduates or above (Table 1).

Table 1: Demographic Characteristics of Participants (n=130)

Demographic Variables	RAS Group (n=65)	Control Group (n=65)
Gender		
Male	47 (72.3%)	46 (70.8%)
Female	18 (27.7%)	19 (29.2%)
Age Group		
18–30 Years	29 (44.6%)	31 (47.7%)
31–45 Years	24 (36.9%)	23 (35.4%)
46–60 Years	12 (18.5%)	11 (16.9%)
Residence		
Urban	41 (63.1%)	39 (60.0%)
Rural	24 (36.9%)	26 (40.0%)
Education Level		
Primary or Less	18 (27.7%)	16 (24.6%)
Secondary	27 (41.5%)	29 (44.6%)
Graduate or Above	20 (30.8%)	20 (30.8%)

The results show significantly higher mean cortisol levels in the RAS group (459.2 ± 49.6 nmol/L) compared to the healthy controls (232.4 ± 119.4 nmol/L, p=0.001). Conversely, ferritin (58.9 ± 7.8 ng/mL vs 80.4 ± 9.7 ng/mL), iron (75.9 ± 13.1 ng/mL vs 85.1 ± 16.2 ng/mL), and folic acid (5.7 ± 3.5 ng/mL vs 8.7 ± 5.7 ng/mL) levels were significantly lower in the RAS group (p=0.001 for all). Vitamin B12 levels were slightly lower in the RAS group (262.0 ± 112.2 pg/mL) compared to healthy controls (279.3 ± 112.6 pg/mL), but the difference was not statistically significant (p=0.079). These findings indicate significant biochemical differences in RAS patients, except for vitamin B12 (Table 2).

Table 2: Comparison of Biochemical Parameters Between RAS and Healthy Control Groups Using Independent Sample t-Test

Variables	RAS Group (Mean ± SD)	Control Group (Mean ± SD)	95% CI	p-value
Cortisol (nmol/L)	459.2 ± 49.6	232.4 ± 119.4	(191.5, 266.7)	0.001
Ferritin (ng/mL)	58.9 ± 7.8	80.4 ± 9.7	(-24.7, -17.2)	0.001
Iron (ng/mL)	75.9 ± 13.1	85.1 ± 16.2	(-13.7, -5.9)	0.001
Folic Acid (ng/mL)	5.7 ± 3.5	8.7 ± 5.7	(-4.5, -1.5)	0.001
Vitamin B12 (pg/mL)	262.0 ± 112.2	279.3 ± 112.6	(-35.5, 0.8)	0.079

The hematocrit was significantly lower in the RAS group (37.88 ± 8.14%) compared to the control group (40.85 ± 5.81%, p=0.018). Red blood cell (RBC) count was lower in RAS patients (4.90 ± 0.76 ×10^6/µL) than in healthy controls (5.21 ± 1.02 ×10^6/µL). Conversely, WBC count was higher in the RAS group (9.73 ± 2.81 ×10^3/µL). Platelet counts were also slightly lower in the RAS group (234.80 ± 56.30 ×10^3/µL) compared to healthy controls (250.84 ± 105.54 ×10^3/µL). These findings suggest significant reductions in hemoglobin and hematocrit levels in RAS patients, while other hematological parameters showed trends toward variation without statistical significance (Table 3).

Table 3: Comparison of Hematological Parameters Between RAS and Healthy Control Groups Using Independent Sample t-Test

Variables	RAS Group (Mean ± SD)	Control Group (Mean ± SD)	95% CI	p-value
Hemoglobin (g/dL)	12.9 ± 2.1	14.0 ± 1.4	(-1.6, -0.5)	0.001
Hematocrit (%)	37.88 ± 8.14	40.85 ± 5.81	(-5.4, -0.5)	0.018
RBC Count (×10^6/µL)	4.90 ± 0.76	5.21 ± 1.02	(-0.6, 0.0)	0.054
WBC Count (×10^3/µL)	9.73 ± 2.81	7.38 ± 1.79	(-0.1, 4.7)	0.057
Platelet Count (×10^3/µL)	234.80 ± 56.30	250.84 ± 105.54	(-45.3, 12.1)	0.282

Among male, cortisol levels were significantly higher in the RAS group (469.2 ± 51.1 nmol/L) compared to healthy controls (240.8 ± 122.3 nmol/L, p=0.0037). Ferritin, iron, folic acid, and vitamin B12 levels showed no statistically significant differences between RAS and control males. In females, folic acid levels were significantly lower in the RAS group (5.5 ± 3.7 ng/mL) than in healthy controls (8.5 ± 3.8 ng/mL, p=0.023), while other biochemical parameters showed no significant variations. Regarding hematological parameters, females with RAS had significantly lower hemoglobin (12.7 ± 2.2 g/dL) than their healthy counterparts (13.8 ± 1.3 g/dL, p=0.001), as well as significantly lower hematocrit levels (37.2 ± 3.8% vs 40.5 ± 3.4%, p=0.004). However, differences in red blood cell (RBC) count, white blood cell (WBC) count, and platelet count were not statistically significant in either gender. These findings highlight gender-specific variations in biochemical and hematological parameters between RAS patients and healthy controls, with significant differences in cortisol levels among males and folic acid, hemoglobin, and hematocrit levels among female (Table 4).

Table 4: Hematological and Biochemical Parameters Between RAS and Healthy Control Groups Stratified by Gender

Variables	Gender	RAS Group (Mean ± SD)	Control Group (Mean ± SD)	95% CI	p-value
Cortisol (nmol/L)	Male	469.2 ± 51.1	240.8 ± 122.3	(25.8, 442.6)	0.037
	Female	448.9 ± 48.5	223.4 ± 116.5	(21.4, 415.4)	0.023
Ferritin (ng/mL)	Male	60.2 ± 7.6	83.1 ± 10.3	(-1.2, 49.4)	0.091
	Female	57.6 ± 8.1	78.2 ± 9.2	(-0.9, 35.8)	0.001
Iron (ng/mL)	Male	77.1 ± 12.8	87.3 ± 17.2	(-1.0, 21.2)	0.065
	Female	74.4 ± 13.4	83.9 ± 15.8	(-0.8, 20.6)	0.078
Folic Acid (ng/mL)	Male	5.9 ± 3.4	9.0 ± 5.6	(-0.6, 6.4)	0.071
	Female	5.5 ± 3.7	8.5 ± 5.8	(-0.3, 5.9)	0.023
Vitamin B 12 (pg/mL)	Male	266.5 ± 110.8	281.9 ± 114.1	(-1.9, 31.5)	0.087
	Female	257.3 ± 113.4	276.4 ± 111.2	(-1.3, 37.4)	0.024

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DISCUSSION

This study demonstrated that patients with recurrent aphthous stomatitis (RAS) had significantly elevated serum cortisol levels and reduced concentrations of ferritin, iron, folic acid, hemoglobin, and hematocrit compared to healthy controls. These findings suggest a multifactorial pathogenesis involving psychological stress, micronutrient deficiencies, and mild hematologic alterations. The most prominent biochemical finding was the elevated cortisol level in RAS patients, supporting the strong link between psychological stress and oral ulcer recurrence. Cortisol, a well-established biomarker of stress, is elevated in multiple studies involving RAS patients [13, 14]. Chronic stress may downregulate immune responses, delay epithelial healing, and increase susceptibility to mucosal breakdown [13]. Current results align with those of Vandana & Kavitha, and Aslam *et al.*, reinforcing the relevance of stress as a central trigger in RAS pathophysiology [14, 15]. Nutritional factors also appear to play a key role. The significantly lower levels of ferritin, serum iron, and folic acid in the RAS group are consistent with previous studies that identified hematinic deficiencies as common in RAS patients [16, 17]. Iron and folate are essential for epithelial regeneration, DNA synthesis, and immune function. Their deficiency may lead to delayed healing and recurrent mucosal injury. Furthermore, reduced hemoglobin and hematocrit levels indicate compromised oxygen transport and tissue repair capacity, potentially exacerbating ulcer formation [17, 18]. Although vitamin B12 levels were slightly lower in the RAS group, the difference was not statistically significant, suggesting a limited role in this population. Gender-specific analysis revealed that female with RAS had significantly lower folic acid, hemoglobin, and hematocrit levels compared to healthy female, potentially due to menstrual losses and hormonal influences. Conversely, male with RAS exhibited significantly elevated cortisol levels, suggesting a stronger stress-related physiological response [19]. These findings highlight the importance of considering gender-specific factors in RAS assessment and management. From a clinical and public health perspective, these results underscore the need to integrate nutritional screening and stress evaluation into routine care for RAS patients. Dentists and primary care providers should be vigilant in identifying micronutrient deficiencies and stress-related triggers, particularly in low-resource settings where nutritional inadequacies are prevalent [20, 21]. Incorporating hematinic profiling and psychosocial assessments could guide more comprehensive and effective treatment strategies.

CONCLUSIONS

It was concluded that RAS exhibits distinct changes in hematological parameters, including stress hormone (cortisol), iron metabolism markers (ferritin, folic acid), and blood cell counts (WBC, RBC, Hb). Thus, thorough evaluations in RAS patients, encompassing blood tests and nutritional analyses, are crucial for uncovering contributing factors and tailoring management based on individual predispositions.

Authors Contribution

Conceptualization: SB
Methodology: SB, AQKD, AN, MM
Formal analysis: MW, KS
Writing review and editing: AQKD, AN

All authors have read and agreed to the published version of the manuscript

Conflicts of Interest

All the authors declare no conflict of interest.

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