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Exploring the relationship between stress and aphthous ulcers among Dentistry college students, Anbar university: Histological investigation

Abdul Nasser H. Warwar[1]
MSc of Oral Histology, Member of Teaching Staff at Department of Oral Histology, College of Dentistry, University of Anbar, AL- Ramadi city, Iraq
Mohammed I. Abdullah[2]
MSc of Preventive Dentistry, Member of Teaching Staff at Department of POP, College of Dentistry, University of Anbar, AL-Ramadi city, Iraq
Wesam A. Sami[3]
Master of Dental Public Health, Member of Teaching Staff at Department of POP, College of Dentistry, University of Anbar, AL- Ramadi city, Iraq

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ABSTRACT
Aphthous ulcers usually exhibit a breakdown in the integrity of the epithelial layer. A necrotic center with an infiltration of inflammatory cells is frequently visible in the ulcer. The study aims to establish if there is an association between stress and the frequency or intensity of recurrent aphthous ulcers (RAUs) in this particular group. Understanding the histological features of RAUs could help in developing therapies and management plans that focus on the main processes that cause RAUs to form. Oral ulcer tissue samples were collected from the individuals. Specimens were obtained by a biopsy punch technique with the use of a local anesthetic. The tissue samples were promptly treated with a suitable fixative to maintain tissue structure. Samples were fixed and then subjected to tissue processing, which involved dehydration, disinfection, and embedding in paraffin wax. Tissue sections were thinly sliced using a microtome and placed on glass slides. The slides underwent staining to improve the visibility of the cellular structure. Microscopy was conducted using a light microscope. Examination of tissue samples from participants with RAUs showed numerous important characteristics. Under a microscope, the epithelium was damaged and the top layer had been lost. There were also lymphocytes, macrophages, and neutrophils in the inflammatory infiltrate.

KEYWORDS: Aphthous ulcers, Histological investigation, Stress, Dental student.

INTRODUCTION
Aphthous stomatitis that returns is extremely frequent. The exact reason is unknown; however, it is likely due to a combination of variables such as genetic predisposition, immune system illnesses or abnormalities, and dental hygiene products and preservatives [1]. Many cases of stomatitis start with recurrent aphthous stomatitis, which typically happens in childhood [2]. Interestingly, statistics show that 80% of those infected with this condition are under the age of 30. Canker sores reoccur often in patients with recurrent aphthous stomatitis [3]. Occasionally throughout the year, a few of them may experience the emergence of an ulcer or two [4]. Although some people experience the ongoing development of ulcers, Age is associated with a decline in both the frequency and intensity of attacks [5]. While many things can put a patient at risk of infection or set off an attack, allergies don't seem to be one of them. Some of these causes involve psychological distress and oral trauma; for instance, a college student can get canker sores right before final exams [6]. In addition to consuming specific foods, such as peanuts, chocolate, coffee, eggs, cereals, almonds, strawberries, cheese, and tomatoes [7]. Massive, long-lasting canker sores in the mouth are a common symptom of AIDS [8]. Canker sores are less common in pregnant women, women who use

birth control pills, and cigarette users, however the exact reason for this is unclear [9]. Any area of loose soft tissue is susceptible to canker sores, including the interior of the mouth, cheeks, tongue, floor of the mouth, soft palate, and throat. Sores have a crimson border and a grayish-yellow core; they can be shallow, circular, or oval in shape [10]. It is thought that the following factors contribute to the development of mouth ulcers: lowered resistance to disease, diminished immunity, psychological strain, and mental health issues [11]. On most occasions the ulcers typically manifest in clusters of two or three and have a diameter of 0.3-0.95 cm. The ulcers often go away without treatment in about a week, and they never leave scars. Although the ulcers are somewhat large, measuring approximately ½ to 1½ inches (less than 3 cm) in diameter, they do not occur very frequently. Large ulcers are typically scar-prone, have an uneven shape, and take many weeks to heal [12]. The purpose of study to understanding the histological features of RAUs could help in developing therapies and management plans that focus on the main processes that cause RAUs to form.

MATERIAL AND METHOD

The study sample was students of the College of Dentistry in Anbar university who suffer from the appearance of these ulcers in the mouth. The questionnaires contained validated instruments from the perceived stress scale (PSS). The patients were requested to complete an anxiety inventory (PSS) in order to evaluate their anxiety and depression levels. The PSS consists of 10 questions, with each response being rated on a scale ranging from 0 to 4. Greater total scores reflect increased severity of anxiety symptoms. Scores between 0 and 13 are classified as low stress. Scores between 14 and 26 indicate moderate stress. Perceived stress levels between 27 and 40 are classified as high. The current Perceived Stress Scale (PSS) score is 29, suggesting that the student is under high levels of perceived stress. The participants completed the questionnaires truthfully and to the best of their abilities. The diagnosis of RAUS was made using clinical criteria, specifically the occurrence of recurrent painful mouth sores. Individuals who met the diagnostic criteria for RAU were chosen for histopathological examination. Histological analysis method: Oral ulcer tissue samples were collected from specific participants. Specimens were obtained using a biopsy punch while the patient was under local anesthesia. The tissue samples were promptly fixed in a suitable fixative (formalin), to maintain tissue structure. Samples were fixed and then processed through dehydration, disinfection, and embedding in paraffin wax. Tissue sections were thinly sliced using a microtome and placed on glass slides. The slides underwent staining, specifically hematoxylin and eosin (H&E) staining, to improve the visibility of cellular features. Microscopy was conducted utilizing a light microscope. Experienced histopathologists investigated and documented the histological characteristics of RAUs, including epithelial features, inflammatory cell infiltration, and ulcer characteristics. The data were coded, validated, and analyzed by SPSS (version 20). Chi-square tests were utilized for the analysis. A probability level below 0.05 was deemed statistically significant.

RESULTS

Histological examination of tissue samples from participants with RAUs showed various important characteristics. Microscopic analysis revealed ulcerated epithelium with loss of superficial layers and a combination of lymphocytes, macrophages, and neutrophils in the inflammatory infiltrate. Furthermore, some samples showed vascular congestion and fibrinopurulent exudate. The histology findings align with the traits and features of RAUs as shown in Figure (1-2).

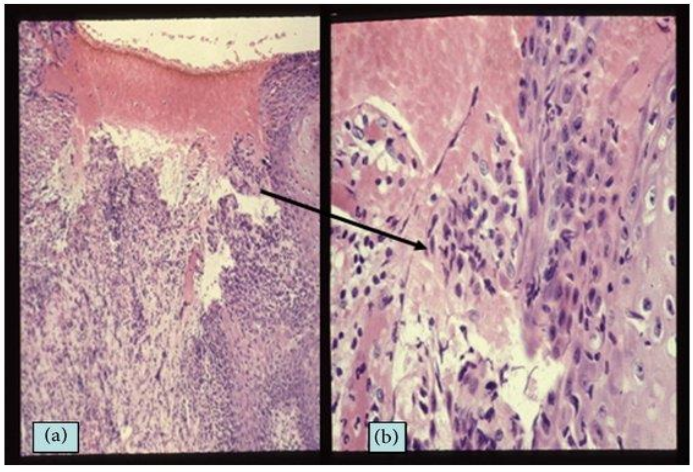

Figure 1 *((a,b): histological findings with the characteristic features of RAUs.)*

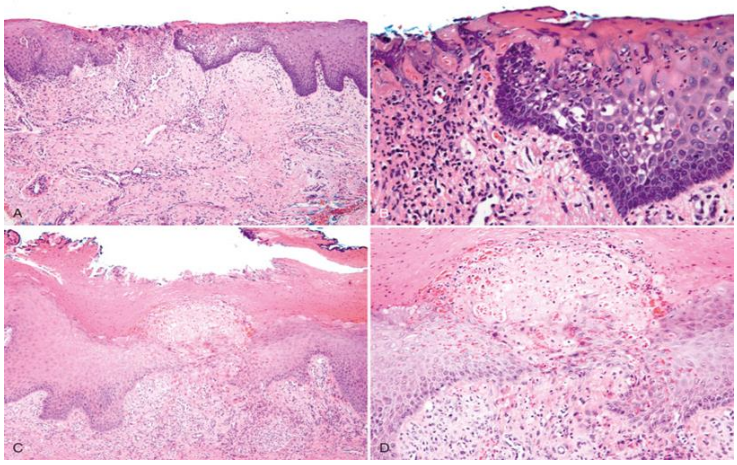

Figure 2 *(Ulcerative and inflammatory condition.)*

Table 1 displays the distribution of recurrent aphthous ulcers among dental students. Among 750 pupils, 35 (4.7%) reported having ulcers, while the remaining 715 students (95.3%) did not. The research indicates that recurrent aphthous ulcers are not common among dentistry students. Table (2) offers a detailed analysis by investigating the distribution of aphthous ulcers based on gender. Out of the thirty-five students who reported having ulcers, 42.9% were men (fifteen students) and 57.1% were women (twenty students). Based on the findings, women may have a higher likelihood of experiencing recurring aphthous ulcers compared to men. Statistically no significant differences between them P > 0.05. Table 3 examines the distribution of recurring aphthous ulcers among different dentistry college classes. Out of the thirty-five pupils with ulcers, fourteen (40%) are from class 5, and thirteen (37.1%) are from class 1. Fewer pupils in classes 2, 3, and 4 reported having ulcers, with percentages of 8.6%, 8.6%, and 5.7%, respectively. Table 4 lists the characteristics and causes of aphthous ulcers. The table emphasizes aspects such as frequency, number of episodes, duration, location inside the oral cavity, and association with other conditions or stress. Most individuals (33) stated that they had ulcers for a duration of seven days. The lip was the most frequent location for ulcers (25), with exam-related stress being the most common relationship (30). The findings illuminate the patterns and characteristics of recurring aphthous ulcers in the studied group.

Table 1 *(distribution of recurrent aphthous ulcer among dental students.)*

Experience ulcer	Number	%
Yes	35	4.7
No	715	95.3
Total	750	100

Table 2 *(The distribution of aphthous ulcer according to gender.)*

Experience ulcer	Male No (%)	Female No (%)	Total No (%)	P (Chi-Squre)
	15 (42.9)	20 (57.1)	35 (100)	0.231*

Table 3 *(The distribution of recurrent aphthous ulcer according to stages of dentistry college.)*

stages	Number	%
Stage 1	13	37.1
Stage 2	3	8.6
Stage 3	3	8.6
Stage 4	2	5.7
Stage 5	14	40
Total	35	100

Table 4 *(Reasons connected to aphthous ulcer.)*

	experience with aphthous ulcer	**Number (%)**

Count of every episodes	1	15
	3-6	20
Duration of ulcer	7 days	33
	7 - 14days	2
Site in the oral cavity	Lip	25
	Cheek	10
relation with stress	Examination	30
	Other	5
Size	Less than 5mm	20
	=5mm	10
	More than 5mm	5
pain	Slight	0
	Moderate	30
	sever	5

DISCUSSION

No previous studies have focused on the histological characteristics of aphthous ulcers among dental students in Iraq, primarily in the College of Dentistry at the University of Anbar, despite abundant literature on their global occurrence. The aim of this study is to address this research gap and improve our comprehension of the subject. Analysis of the histopathological features of RAUs: The histological examination of tissue samples from participants with recurrent aphthous ulcers showed traits and features that align with findings from other investigations. The ulcerated epithelium with loss of superficial layers, mixed inflammatory infiltration, vascular congestion, and exudate are consistent with the established histological features of RAUs (13). The tissue alterations seen in RAUs are constant throughout various populations, including dental students. Females in the research had a significantly higher occurrence of recurring aphthous ulcers (57.1%) than males (42.9%), with a p-value of less than 0.001. This discrepancy was greater than what was observed in studies by Handa et al. (14), Santosh Patil et al. (15), and Safadi (16). Women are more prone to stress and emotional situations that might impact their immune system, and hormonal changes during pregnancy and menstruation can also be a factor (17). The research noted a greater occurrence of ulcers among dental students in their first and final years of education, with rates of 37.1% and 40% respectively, surpassing the results reported by George and Joseph (18) and Singh et al. (19). First-year students face increased stress due to the transition from school to formal education and the challenging curriculum. Final-year students have a higher prevalence of stress due to the demanding nature of their studies. The research methodology said that the stress intensity scale showed a high level of tension, with a score of 29. 30 participants in the study experienced stress-related ulcers, notably during tests, suggesting the influence of stress on the occurrence of recurrent aphthous ulcers, especially in those with underlying anxiety characteristics (20,21). Kasi et al. (2007) discovered that medical graduates faced high levels of stress, impacting their stress management by resorting to poor coping strategies (22). Stress and RAUs connection analysis: Our study found a notable connection between stress levels and the presence or seriousness of RAUs in dentistry students. Participants with elevated perceived stress levels exhibited a greater occurrence and more intense episodes of RAUs. Consistent with previous research, this finding supports the notion that stress plays a role in the development or worsening of RAUs (23, 24). It is essential to include psychological factors when managing and preventing RAUs in dental students due to the strong connection between stress and RAUs. Some investigations have shown conflicting results, suggesting a weak or absent connection between stress and RAUs (24). Differences in the study population, stress assessment methods, or other influencing factors may be responsible for these discrepancies.

Limitations of the study

Considering various constraints is essential while analyzing the study's findings. The cross-sectional approach initially limits the ability to show a causal association between RAUs and stress. Longitudinal studies would offer stronger evidence on the chronological connection between stress and the development or seriousness of RAUs. Furthermore, our study did not completely consider confounding factors such food patterns, dental hygiene practices, and genetic predispositions. Future research should focus on overcoming these limitations by using more thorough study designs and accounting for potential confounding variables.

Clinical Implications

Researchers believe that multiple factors, sometimes even in the same individual, contribute to outbreaks of mouth ulcers, although the exact cause is yet unknown. Minor oral injuries from dental procedures or over-brushing are two potential causes of mouth ulcers. Brushing with sodium lauryl sulfate-containing toothpaste and mouthwash as

recommended. Consuming food that is deficient in vitamin-containing components. B12, iron, folic acid, zinc.

CONCLUSION

We conclude from this study conducted on dental students in Ramadi city that psychological pressure has a significant impact on increasing the rate of cortisone, which has a significant impact on lowering immunity, and thus the mouth is vulnerable to the occurrence of these ulcers. Microscopy was conducted using a light microscope. Examination of tissue samples from participants with RAUs showed numerous important characteristics. Under a microscope, the epithelium was damaged and the top layer had been lost. There were also lymphocytes, macrophages, and neutrophils in the inflammatory infiltrate.

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