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Salivary TNF and Blood Monocytes in Recurrent Aphthous Ulceration: Insights from Clinical and Immunological Evaluation

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ABSTRACT

Rise levels of TNF have been detected in the tissues, saliva, and blood of patients with recurrent aphthous stomatitis, suggesting its participation in the progress and persistence of these ulcerative lesions. This study aims to examine the levels of tumor necrosis factor in the saliva of people who have recurrent aphthous stomatitis in order to learn more about its pathophysiology. The study involved 80 individuals, including 40 patients identified with RAU and 40 sex- and age-matched healthy individuals as controls. TNF levels were estimated by using commercially available micro-well enzyme-linked immunosorbent assay kits; monocytes were determined by using an automated hematological analyzer XT2000i (from Sysmex, Japan). The statistical analysis revealed a significant increase in TNF and monocyte count increased significantly in patients with aphthous ulcers when compared with healthy individuals (p-value < 0.000). Also, TNF was significantly higher in major aphthous ulcers compared to herpetiform aphthous ulcers, but statistical analysis revealed no significant difference between minor aphthous ulcers and herpetiform aphthous ulcers.

Keywords: Aphthous, TNF, Ulceration, WBC

1 Introduction

IN response to infection or inflammation, macrophages and T lymphocytes mainly create Tumor Necrosis Factor (TNF), a cytokine that promotes inflammation. TNF significantly impacts apoptosis (programmed cell death), inflammation, and immune response regulation [1]. Tumor suppression and immune system regulation are two other roles for TNF. A main goal for pharmacological inhibitors, however, is that TNF overproduction is connected to chronic inflammatory disorders, including inflammatory bowel disease, psoriasis, and rheumatoid arthritis [2, 3]. Recurrent Aphthous Stomatitis (RAS), also referred to as canker sores, is a prevalent oral mucosal condition marked by pain and ulceration [4, 5]. The etiology of RAU is ambiguous; nevertheless, research on pro-inflammatory cytokines, particularly TNF, indicates a substantial involvement in its pathogenesis [6. 7]. Rise levels of TNF have been detected in the tissues, saliva, and blood of patients with RAS, suggesting its participation in the progression and persistence of these ulcerative lesions [8]. This study aims to examine the levels of Tumor Necrosis Factor in the saliva of people who have Recurrent Aphthous Stomatitis in order to learn more about its pathophysiology. In order to further understand any systemic inflammatory reactions linked to RAS, the study also aims to measure blood monocyte count. The goal of this research is to better understand the immunopathological mechanisms of RAS and to evaluate the possibility of these parameters as investigative or

prognostic indicators for disease severity and recurrence by examining them.

2 Materials and Methods

From the beginning of January until the end of February, researchers in Diyala conducted this case-control study. To find out what factors can lead to chronic aphthous ulcers, the researchers compared the medical records of affected patients with those of healthy controls. Forty patients with RAU and forty healthy people matched for sex and age served as the study's 80 participants. Members worked at various academic medical centers. Before anyone could participate, they had to sign an informed consent form stating that they were willing to give full clinical and laboratory data and would cooperate fully throughout the study. Either healthy people without a history of recurrent aphthous ulcers (RAU) or those with a diagnosis of RAU were eligible to participate. Those who had a history of smoking, high blood pressure, or diabetes, or who had recently used anti-inflammatory medicine, were not eligible. People with chronic disorders or oral infections were also not included. Five to ten milliliters of unstimulated saliva were collected from each participant in the present study. In order to guarantee high-quality specimen collection, everyone was told to refrain from eating for at least an hour before the operation and to avoid chest stimulation in the time leading up to saliva collection. The saliva samples were quickly frozen and sent to Al-Shams Medical Laboratory for analysis after collection. Before the TNF test, some samples were separated by using a centrifuge at 5000 rpm for 15 minutes and stored at -20°C. The other samples were transferred to EDTA tubes for direct white blood cell measurement. We used commercially available micro-well enzyme-linked immunosorbent assay kits to measure TNF levels. Using an automated hematological analyzer XT2000i (from Sysmex, Japan), the monocyte count in the whole blood of all individuals was determined.

SPSS 24 (Chicago, Illinois, USA) was the primary tool for all statistical studies. Percentages, averages, and standard errors were computed as descriptive statistics. The Unpaired T-test was one of the inferential statistics tests used to compare group means. The TNF and leukocyte counts were compared among the three groups using one-way ANOVA. For all analyses, a p-value less than 0.05 was considered statistically significant.

3 Results

The study's individual distribution is displayed in Table 1. The highest number of instances, accounting for 27.5% of all cases, occurred in patients with aphthous ulcers in their 40s, with ages ranging from 24 to 87 (mean ± SD = 49.00 ± 15.18) (Figure 1).

TABLE 1. Age distribution of 80 study individuals according to 10-year age intervals.

Healthy individual			Aphthous ulcers	
Age Interval (years)	No.	%	No.	%
20-29	5	12.5	4	10
30-39	11	27.5	10	20
40-49	8	20	6	15
50-59	8	20	11	27.5
60-69	4	10	5	12.5
70-79	4	10	3	7.5
>80	---	---	1	2.5
Total	40	100	40	100
Mean Age ± SD	46.97 ± 15.18		49.00 ± 15.18	
Median	45.5		50	
Range	24-78		24-87	
Gander				
Male	17	42.5	17	42.5
Female	23	57.5	23	57.5
Total	40	100	40	100

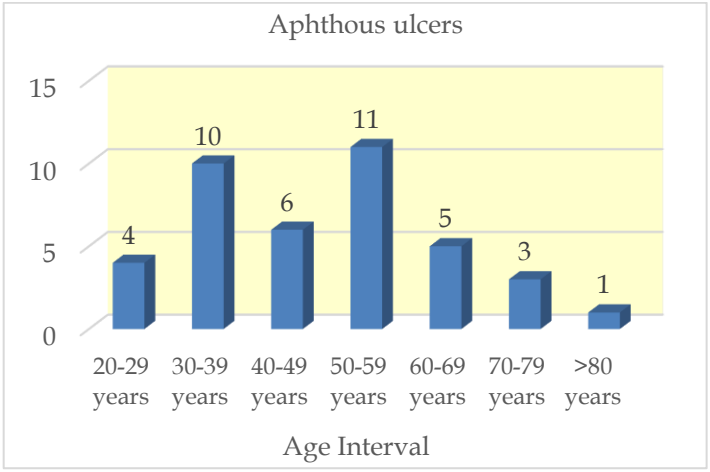

Fig. 1. Distribution of age in Aphthous ulcers according to 10-year intervals.

Seventeen patients (42.5%) were male, and the remaining 23 (47.5%) were female (Figure 2).

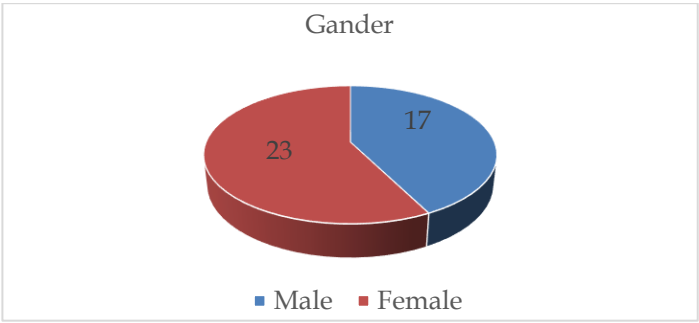

Fig. 2. Distribution of 40 Aphthous ulcer cases according to gender.

Most of the primary sites were found in the buccal mucosa (27 instances, or 67.5% of the total), followed by the tongue (11 cases, or 27.5% of the total) and finally, the lip and floor of the mouth (one case, or 2.5%), Table 2 and Figure 3.

TABLE 2. Site distribution of 40 Aphthous ulcer cases.

Site	No.	%
Buccal mucosa	27	67.5
Tongue	11	27.5
Lip	1	2.5
Floor of the month	1	2.5
Total	40	100

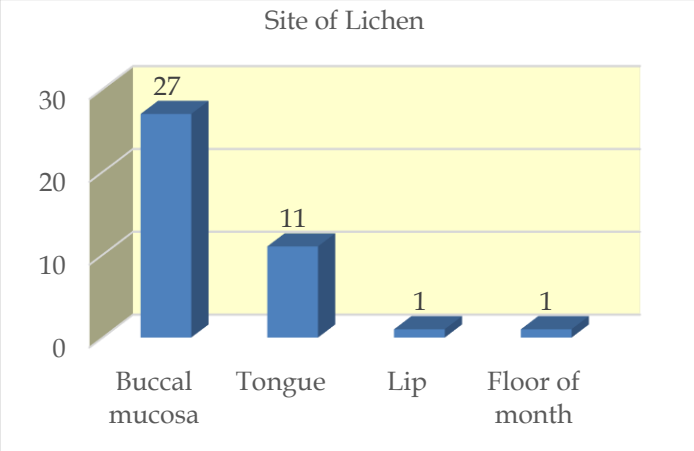

Fig. 3. Distribution of 40 Aphthous ulcer cases according to site.

Aphthous ulcers of the minor variety were the most common (20 cases, or 50%), followed by those of the major variety (15 cases, or 37.5%), and those of the herpetiform variety were the least common (5 cases, or 12.5%), Table 3 and Figure 4.

The statistical analysis revealed a significant increase in TNF, and monocyte count increased significantly in patients with aphthous ulcers (35.32 ± 1.51 ng/l and 0.78 ± 0.02 × 10^9/L) when compared with healthy individuals (19.53 ± 1.19 ng/l and 0.42 ± 0.02 × 10^9/L) (p-value < 0.000) (Table 4).

TABLE 3. Type distribution of 40 Aphthous ulcer cases.

Type	No.	%
Minor aphthous ulcers	20	50
Major aphthous ulcers	15	37.5
Herpetiform aphthous ulcers	5	12.5
Total	40	100

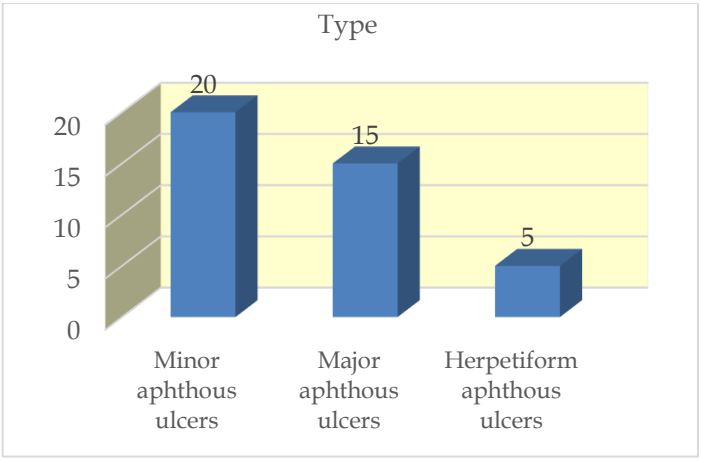

Fig. 4. Distribution of 40 Aphthous ulcer cases according to type.

TABLE 4. Comparative TNF and monocyte count among 40 healthy individuals and 40 Aphthous ulcer cases.

Parameters	Healthy individual Mean ± SE	Aphthous ulcers cases Mean ± SE	t-test p-value
TNF ng/l	11.45 ± 0.37	19.70 ± 0.37	<0.000
Monocytes ×10^^{9}/L	0.42 ± 0.02	0.78 ± 0.02	<0.000
*p-value significant < 0.05; SE. Standard error, TNF. Tumor necrosis factor			

According to the site of the aphthous ulcer, no statistically significant change in TNF and monocyte count was found between the buccal mucosa (20.71 ± 0.22 ng/l and 0.78 ± 0.02 ×10^9/L) and the tongue (17.90 ± 0.61 ng/l and 0.81 ± 0.03 ×10^9/L) (p-value > 0.05) (Table 5).

TABLE 5. Comparative TNF and monocyte count among the sites of 40 Aphthous ulcer cases.

Parameters	Buccal mucosa Mean ± SE	Tongue Mean ± SE	t-test p-value
TNF ng/l	20.71 ± 0.22	17.90 ± 0.61	0.176
Monocytes ×10^^{9}/L	0.78 ± 0.02	0.81 ± 0.03	0.446
*p-value significant < 0.05; SE. Standard error, TNF. Tumor necrosis factor			

Table 6 illustrates the differences in TNF and monocyte count among different types of aphthous ulcers. The mean TNF was significantly higher in major aphthous ulcers (22.42 ± 1.80 ng/L) compared to herpetiform aphthous ulcers (14.91 ± 3.09 ng/L) (p-value = 0.048) but not significant with the minor group (18.86 ± 1.62 ng/L) (p-value = 0.152), as well as statistical analysis did not show a significant difference between minor aphthous ulcers and herpetiform aphthous ulcers (p-value = 0.274).

Monocyte count did not find any significant difference among the three groups: major (0.78 ± 0.02 ×10^9/L), minor (0.78 ± 0.03 × 10^9/L), and herpetiform (0.78 ± 0.04 × 10^9/L) (p-value > 0.05) (Table 6).

4 Discussion

TNF-α significantly mediates acute inflammation, while RAS is a condition of uncertain origin that can lead to considerable morbidity. One or more isolated, superficial, painful ulcers are located on the unattached oral mucosa. Minor aphthous ulcers generally last for 7 to 10 days and resolve without leaving scars. Significant aphthae may persist for several weeks to months and can result in scarring during the healing process [9]. Monocytes are essential in the synthesis and control of Tumor Necrosis Factor-alpha within the innate immune response [10]. Upon activation by infections, tissue injury, or inflammatory stimuli such as lipopolysaccharides from bacterial cell walls, monocytes develop into macrophages and dendritic cells, which are primary producers of TNF [11]. Our results found increased saliva TNF and monocyte count in blood in patients with RAU when compared with healthy people, and increased TNF in major RAU when compared with herpetiform RAU. This indicates an increase in the TNF during the active stage of the disease. Similar findings have been reported by Batool Al-Ghurabei et al. (2011), Boras et al. (2006), and Natah et al. (2000) [12, 13].

In a study by Valle and associates, who studied 20 patients with RAS, they detected that saliva TNF increased significantly in patients with active lesions of RAS compared with healthy people [14]. The role of TNF in the disease's pathogenesis is shown by two explanations: high concentrations of TNF are current through the initial phases of mouth ulcerations, and certain medications, which possess anti-TNF properties, determine significant efficacy in treating recurrent aphthous stomatitis and other ulcerative conditions, including Adamantiades-Behçet disease [15-17]. Salivary TNF may significantly contribute to the pathophysiology of this disease and could also be pivotal in the exploration of novel therapies. These findings suggest a possible role of the oral immune system in the etiology of RAU.

5 Conclusion

Salivary TNF concentrations were significantly higher in RAS patients compared to healthy controls, according to our findings. Our research leads us to believe that measuring levels of TNF in saliva can serve as a trustworthy indicator of RAS advancement or regression. All of this research backs the significance of TNF-α in the development of RAS.

Conflict of Interest: The authors declare no conflict of interest.
Financing: The study was performed without external funding.
Ethical consideration: The study was approved by Diyala University, Diyala, Iraq.

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TABLE 6. TNF and monocyte count among the 40 cases of 40 Aphthous ulcers.

Parameters	Major Mean ± SE	Minor Mean ± SE	Herpetiform Mean ± SE	ANOVA p-value
TNF ng/l	22.42±1.80	18.86 ± 1.62	14.91±3.09	Major vs. Minor 0.152 Major vs. Herpetiform 0.048 Minor vs. Herpetiform 0.274
Monocytes ×10^9/L	0.78 ± 0.02	0.78 ± 0.03	0.78 ± 0.04	Major vs. Minor 0.972 Major vs. Herpetiform 0.925 Minor vs. Herpetiform 0.942
*p-value significant < 0.05; SE. Standard error, TNF. Tumor necrosis factor				

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