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Correlation Between Salivary Interleukin-6 and White Blood Cells in Patients with Recurrent Aphthous Ulceration: A Clinical and Immunological Assessment

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ABSTRACT

Recurrent aphthous ulceration is the predominant oral condition, impacting 5–20% of the general populace, IL-6 is a crucial mediator in signaling acute inflammatory responses generated at infection sites and functioning as a warning signal to the entire organism. This study aims to quantify salivary IL-6 levels in patients with recurrent aphthous ulcers to evaluate the potential local synthesis of cytokines at the disease site. The study involved 80 individuals, including 40 patients identified with RAU and 40 sex and age matched healthy individuals as controls. Interleukin-6 levels were estimated by using commercially available micro-well enzyme-linked immunosorbent assay kits, white blood cells were determined by using an automated hematological analyzers XT2000i (from sysmex, Japan). The statistical analysis revealed a significant increase in IL-6 and white blood cells level increased significantly in patients with aphthous ulcers when compared with healthy individual (p-value <0.000) as well as the mean IL-6 level was significantly higher in major aphthous ulcers compared to minor aphthous ulcers (p-value = 0.005) and herpetiform aphthous ulcers (p-value = 0.008). Nonetheless, statistical analysis revealed no significant difference between minor aphthous ulcers and herpetiform aphthous ulcers.

Keywords: Aphthous, Herpetiform, IL-6, Ulceration

1 Introduction

MOUTH ulcers are dominant oral disease, Understanding the probable mechanisms underlying ulcer development and the effect of clinical conditions facilitates accurate diagnosis, successful treatment, and possibly avoids recurrence [1]. Recurrent aphthous ulceration (RAU) is the primary oral disorder, affecting 5–20% of the general population [2]. The phenomenon observed in RAU is probably initiated by an unidentified exogenous or endogenous antigenic stimulation of keratinocytes, resulting in the secretion of T-cell activation cytokines, particularly interleukin (IL) [3]. The immune response triggered by activated cytotoxic T lymphocytes induces epithelial damage through the cytotoxicity of oral epithelial cells, leading to epithelial loss by direct keratinocyte lysis. Phagocytic mononuclear cells and neutrophilic leukocytes are involved in this immunological damaging mechanism [4]. Histologic surveys indicate that active cell-mediated immunity and localized cytokine making are typical changes in RAU. Various materials, including serum, genetic material, and tissue samples, from patients with RAU, have been observed to clarify cytokine profiles in these individuals, about IL-1, IL-2, IL-3, IL-4, IL-5, IL-6. However, IL-6 may have a important role in the etiology of RAU [4]. IIL6 controls many physiological processes, differentiation, cellular proliferation, and encompassing inflammation [5, 6]. IL-6 is a soluble mediator that influences inflammation,

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immune response, and hematopoiesis in numerous ways. Human IL-6 has 212 amino acids, which includes a 28-amino-acid signal peptide, and is encoded by a gene located on chromosome 7p21. The core protein possesses a molecular weight of approximately 20 kDa, however glycosylation increases its size to 21–26 kDa in its native form [7]. It plays a crucial mediator in signaling acute inflammatory responses produced at infection sites and functioning as a warning signal to the entire organism [8]. This study aimed to measure salivary IL-6 level in patients with recurrent aphthous ulcers to evaluate the likely local production of cytokines at the site of the disease.

2 Materials and Methods

2.1 Study design and duration

This case-control research was performed in Diyala from early January to late February. The study matched clinical and laboratory data from affected patients with healthy controls to investigate potential characteristics associated with recurring aphthous ulcers.

2.2 Study population

The study comprised 80 participants, consisting of 40 patients diagnosed with RAU and 40 healthy persons matched for sex and age as controls. Members were employed from multiple teaching hospitals. Previous to participation, comprehensive informed consent was obtained from all members, confirming their readiness to provide complete laboratory and clinical data to completely cooperate during the study.

2.3 Inclusion and exclusion criteria

Participants were required to be either diagnosed with recurrent aphthous ulcers (RAU) or healthy individuals with no prior history. The exclusion criteria included persons with a smoking history, hypertension, diabetes mellitus, recent anti-inflammatory medication usage, chronic diseases, or oral infections.

2.4 Sample collection and processing

From all individuals involve in current study, 5-10 ml of Unstimulated saliva specimens were taken. all individuals were instructed to avoid chest stimulation prior to saliva collection and to abstain from eating for at least one hour before the procedure to ensure good quality control in specimen collection. After sample collection, the saliva specimen was promptly placed on ice and conveyed to Al-Shams Medical Laboratory for processing. The samples were centrifugation at 5000 rpm for 15 minutes for separation, which was thereafter kept at -20°C until IL-6 assay was perform, other sample but into EDTA tubes for direct determination of white blood cells counts.

2.5 Laboratory analysis

IL-6 levels were quantified using commercially offered micro-well enzyme-linked immunosorbent assay kits. This technique providing a consistent and reliable quantity of IL-6 levels in the found samples, enabling comparison study among patients and healthy subjects. Total white blood cells in whole blood of all subjects were determined by using an automated hematological analyzers XT2000i (from sysmex, Japan).

2.6 Clinical data collection

General clinical data on the sizes and classification of aphthous ulcers were collected through physical investigations of the oral cavity and structured questionnaires accessible to members. This method facilitated a comprehensive assessment of disease presentation and any correlation with salivary IL-6 levels. This study used a severe method to deepen our knowledge of IL-6's role in RAU and to inform upcoming investigative and treatment methods for this dominant oral illness.

2.7 Statistical analysis

All statistical analyses were focused applying the (SPSS), version 24 (Chicago, Illinois, USA). Descriptive statistics were calculated, percentages, means, and standard error. Various tests were employed for inferential statistics, such as the Unpaired T-test for comparing means between two groups. One-way ANOVA was utilized to compare IL-leukocytes count among three groups. A p-value less than 0.05 was thought statistically significant for all analyses.

3 Results

Age distribution of the study sample is shown in Table 1.

TABLE 1. Age distribution of 80 study individual according to 10 years age intervals.

Healthy individual			Aphthous ulcers	
Age Interval	No.	%	No.	%
20-29 years	5	12.5	4	10
30-39 years	11	27.5	10	20
40-49 years	8	20	6	15
50-59 years	8	20	11	27.5
60-69 years	4	10	5	12.5
70-79 years	4	10	3	7.5
>80 years	---	---	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

The peak incidence occurred in the fourth decade of life (11 cases – 27.5 %) with an age range of 24 to 87 years (mean ± SD = 49.00 ± 15.18) in patients with aphthous ulcers (Figure 1).

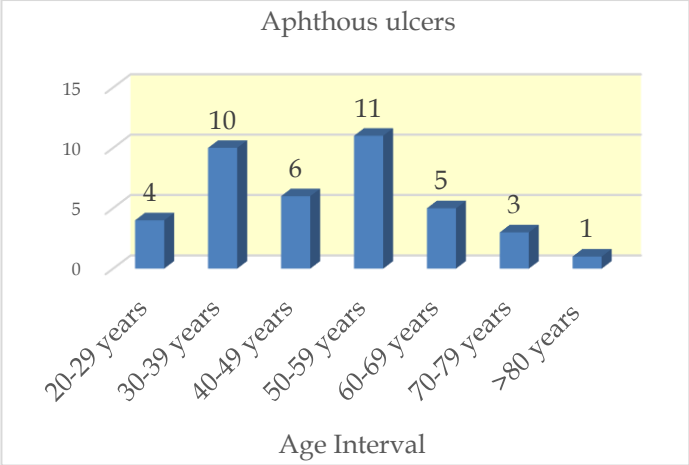

Fig. 1. Distribution of age in Aphthous ulcers according to 10 years intervals.

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

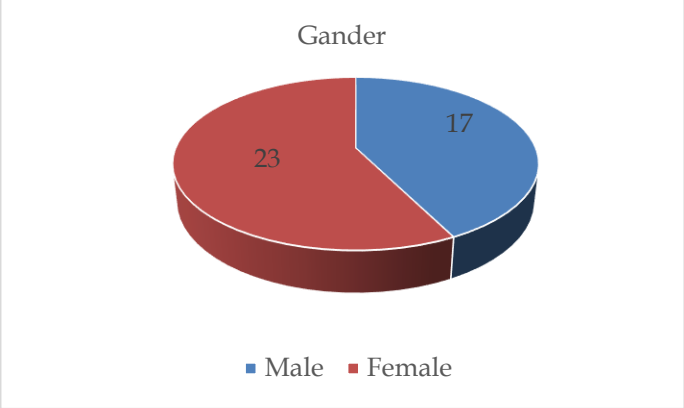

Fig. 2. Distribution of 40 Aphthous ulcers cases according to gender.

Primary sites were mainly identified in the Buccal mucosa (27 cases - 67.5%), followed by the Tongue (11 cases - 27.5%), and only one case was recorded in the lip and floor of the mouth (1 case - 2.5 %) (Table 2 and Figure 3).

TABLE 2. Site distribution of 40 Aphthous ulcers cases.

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

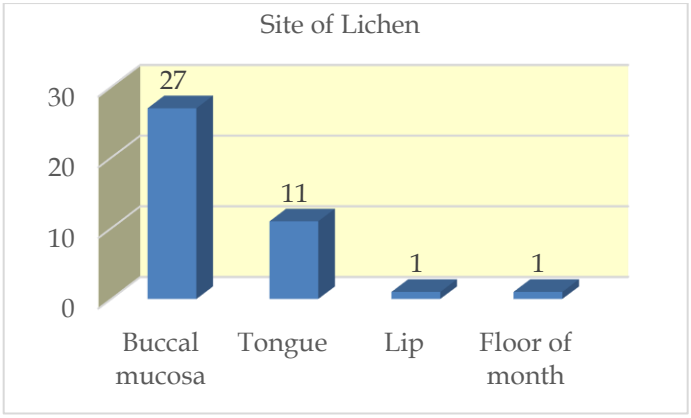

Fig. 3. Distribution of 40 Aphthous ulcers cases according to site.

The most commonly identified type was minor aphthous ulcers (20 cases – 50%), followed by major aphthous ulcers (15 cases – 37.5%), while herpetiform aphthous ulcers were the least frequent (5 cases – 12.5%)(Table 3 and Figure 4).

TABLE 3. Type distribution of 40 Aphthous ulcers cases.

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

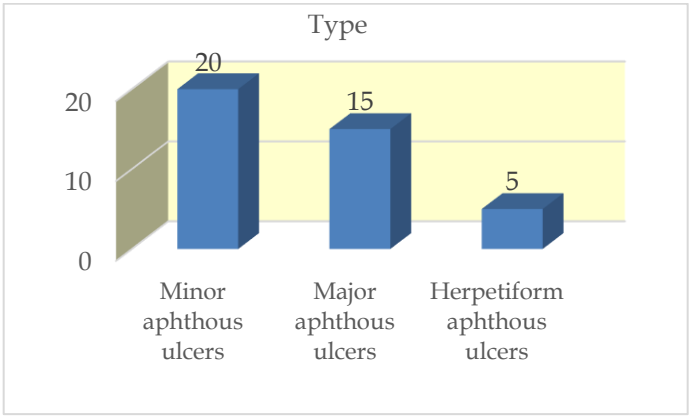

Fig. 4. Distribution of 40 Aphthous ulcers cases according to type.

The statistical analysis revealed a significant increase in IL-6 and white blood cells level increased significantly in patients with aphthous ulcers (35.32 ± 1.51 ng/l, 8.12 ± 0.23 x 10^9/L) when compared with healthy individual (19.53 ± 1.19 ng/l, and 5.44 ± 0.11 x 10^9/L) (p-value < 0.000) (Table 4).

According to the site of the aphthous ulcer, statistical analysis did not find any significant difference in IL-6 and white blood cells levels between the buccal mucosa (34.33 ± 1.81 ng/l, and 8.13 ± 0.22 x 10^9/L) and the tongue (35.17 ± 2.58 ng/l, 7.90 ± 0.22 x10^9/L) (p-value >0.05) (Table 5).

TABLE 4. Comparative IL-6 and WBC among 40 healthy individual and 40 Aphthous ulcers cases.

Parameters	Healthy individual Mean ± SE	Aphthous ulcers cases Mean ± SE	t-test p-value
IL-6 ng/l	19.53 ± 1.19	35.32 ± 1.51	<0.000
WBC x 10^9/L	5. 44± 0.11	8.12 ± 0.23	<0.000
*p-value significant <0.05; SE. Standard error			

TABLE 5. Comparative IL-6 and WBC among site of 40 Aphthous ulcers cases.

Parameters	Buccal mucosa Mean ± SE	Tongue Mean ± SE	t-test p-value
IL-6 ng/l	34.33 ± 1.81	35.17 ± 2.58	0.799
WBC x 10^9/L	8.13 ± 0.22	7.90 ± 0.22	0.668
*p-value significant <0.05; SE. Standard error			

Table 6 illustrates the differences in IL-6 levels among different types of aphthous ulcers. The mean IL-6, level was significantly higher in major aphthous ulcers (41.21 ± 2.01 ng/L) compared to minor aphthous ulcers (32.53 ± 1.85 ng/L) (p-value = 0.005) and herpetiform aphthous ulcers (28.84 ± 5.18 ng/L) (p-value = 0.008). However, statistical analysis did not show a significant difference between minor aphthous ulcers and herpetiform aphthous ulcers (p-value = 0.392). while white blood cells don't show any different significantly among major, minor, and herpetiform (8.31 ± 0.30 x 10^9/L, 7.63 ± 0.44 x 10^9/L, and 8.87 ± 0.35 x 10^9/L respectively).

TABLE 6. Comparative IL-6 and WBC among type of 40 Aphthous ulcers cases.

Parameters	Major Mean ± SE	Minor Mean ± SE	Herpetiform Mean ± SE	ANOVA p-value
IL-6 ng/l	41.21 ± 2.01	32.53 ± 1.85	28.84 ± 5.18	Major Vs. Minor 0.005 Major Vs. Herpetiform 0.008 Minor Vs. Herpetiform 0.392
WBC x 10^9/L	8.31± 0.30	7.63± 0.44	8.87± 0.35	Major Vs. Minor 0.152 Major Vs. Herpetiform 0.182 Minor Vs. Herpetiform 0.446
*p-value significant <0.05; SE. Standard error				

4 Discussion

Interleukin-6 is a pro-inflammatory cytokine play vital for inflammation and immunological responses [9, 10].

Our investigation revealed a significant raise of IL-6 levels in patients with recurrent aphthous ulcers compared to healthy people. The increase of IL-6 recommends a potential role of this cytokine in the start and development of RAU. The findings indicate that IL-6 may be involved in the inflammatory pathways associated with the illness, hence strengthening its correlation with immunological dysregulation in affected patients [11]. The notable difference in IL-6 levels between RAU patients and healthy controls highlights its potential as a biomarker for disease activity and severity.

Raised Il-6 levels have been detected in several inflammatory conditions, including recurrent aphthous stomatitis, referred to as recurrent aphthous ulcers [10]. A systematic review and meta-analysis verified that RAU patients had significantly rise salivary IL-6 level compared to healthy people, demonstrating a systemic inflammatory response related with the disorder. Research indicates that salivary IL-6 levels are significantly elevated during active RAS episodes and decrease following treatment, suggesting that salivary IL-6 may serve as a potential biomarker for evaluating disease activity [12-14].

Cytokines exert an antiregulatory function in immunological responses and various other activities. Based on their structure and function [11, 15, 16]. This study assessed and contrasted the levels of IL-6 in patients with RUA and healthy individuals. Interleukin-6 (IL-6) is primarily implicated in the immune response and the modulation of inflammation. Research indicates that elevated IL-6 levels in RUA patients historically exacerbate the inflammatory response and facilitate the worsening of ulcers [17]. In this study, the contents of cytokines IL-6, IL-8 and TNF-a in the RAU group were significantly higher than in healthy individuals. The results of this study also indicate that IL-6 -a play an important role in the pathogenesis of RAU patients [18, 19].

5 Conclusion

RAU formation and recurrence are associated with elevated systemic and local IL-6 levels. Our results, which highlight IL-6's significance as a possible biomarker and therapeutic target, make improved diagnostic and therapeutic options for RAS patients possible.

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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