Estimation of Interleukin 8 in Aphthous Ulceration

Arjwan M. Shuker¹, Wael T. Al-Wattar², Mahmoud Y.M. Taha¹,*

¹Department of Dental Basic Sciences, College of Dentistry, Mosul University, IRAQ
²Department of Oral Surgery, College of Dentistry, Mosul University, IRAQ
*Corresponding author: tahadent04@yahoo.com

Received November 11, 2021; Revised December 14, 2021; Accepted December 22, 2021

Abstract

Recurrent aphthous ulceration (RAU) is an acute inflammatory disease affecting oral mucosa. It has been reported that cytokines such as IL2, TNF α, IL6 were produced in patients with RAU. IL8 is a neutrophil chemotactic factor and its level increases in response to virus and bacterial products and TNF α or IL1β production.

The aim of the study was to estimate the concentration of IL-8 in RAU patients compared to control group. The study was carried out on a total number of 70 individuals of both sexes and different age groups (50 patients and 20 control) attending Department of Oral Surgery, Unit of Oral Medicine, College of Dentistry /Mosul University for the period from 1/10/2017 to 1/4/2018. Clinical examination was performed and blood samples collected, serum was separated and used in ELISA test to estimate IL-8 concentration. The location of RAU was common in the lower lips followed by buccal mucosa. The concentration of IL-8 in RAU patients was highly significant compared to control group. Within different age groups, IL-8 was higher in age group 13-30 years old. Also high concentration of IL-8 was noticed in females more than males. Minor RAU showed higher concentration of IL-8.

Keywords: RAU, IL-8

Cite This Article: Arjwan M. Shuker, Wael T. Al-Wattar, and Mahmoud Y.M. Taha, “Estimation of Interleukin 8 in Aphthous Ulceration.” International Journal of Dental Sciences and Research, vol. 9, no. 2 (2021): 37-41. doi: 10.12691/ijdsr-9-2-4.

1. Introduction

Recurrent aphthous ulceration (RAU) is acute inflammatory disease affecting oral mucosa. Three forms of RAU exist; minor RAU, major RAU and herpetiform RAU depending on the size and number of ulcers, duration of healing and the ability to heal without scar formation [1]. The prevalence of RAU worldwide is about 20% [2]. It is more common in females with age 2-17 years [3].

The predisposing factors for RAU include sex, hematological and hormonal disturbances, stress, psychological profile, ceasing smoking, trauma and hereditary oxidative stress [4].

The etiological factors of RAU include irritating agents, hypersensitivity to food products, trauma, bacteria and viruses [5].

It has been reported that cytokines such as IL2, TNF α, IL6 are produced from circulating mononuclear cells in patients with RAU [5,6]. Cytokines are small secreted proteins that mediate inflammation and immune response. IL8 is a neutrophil chemotactic factor (NCF) [7]. Its level increases in response to virus and bacterial products and TNF α or IL1β production [8].

This study was conducted to estimate the level of IL8 in RAU patients in relation to sex, age groups, locations compared to control group in Mosul.

We studied RAU from different aspects like bacteriological, biochemical and immunological and this research continues our project on RAU.

2. Materials and Methods

2.1. Study Groups Selection

This study was carried out on a total number of 70 individuals attending Department of Oral Surgery, Unit of Oral Medicine, College of Dentistry /Mosul University for the period from 1/10/2017 to 1/4/2018. Control group consisted of 20 healthy individuals of both sexes with ages range between 20-40 years old. This group had no signs of any systemic disease, or any types of aphthous ulceration in the oral cavity. The study group consisted of 50 individuals aged between 13-62 years old, 24 males and 26 females. In the clinical examination, the patients revealed the presence of RAU according to the recommended criteria [9,10] a. Samples were collected from patients with the classic history and appearance of RAU, b. The size of the lesions varied from 1 mm to 1 cm. Multiple ulcers were also observed in some patients, c. Information was obtained from each patient according to case sheet which included (gender, age, location, color, edge, family history and recurrence of lesions), d. The patients should not be under chronic systemic diseases, e. Drug therapy in the week prior to the examination and recent dental treatment, f. With no history of RAU served as controls group, they all had healthy oral mucosa.
2.2. Blood Collection and Serological Test

Blood sample of about 5ml was taken from each subject and collected in a sterile plastic tube, centrifuged at 3500 rpm for 10 minutes and the serum was separated and placed in a sterile plastic Eppendorff tubes (0.5ml) and stored at (-20˚C) in a deep freeze until the time of analysis. ELISA assay was used to detect IL8 in the serum of control and study groups supplied by Boster, China. Principle of the method is based on the standard sandwich enzyme-linked immune-sorbent assay technology. Human IL8 specific monoclonal biotinylated antibodies were precoated onto 96-well plates. The test samples and biotinylated detection antibodies were added to the wells which is then followed by washing with phosphate buffer saline (PBS). Avidin-Biotin-Peroxidase Complex (ABC) was added and unbound conjugates were washed with PBS. Tetra methyl benzidine (TMB) was used to visualize enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution(2N sulfuric acid). The density of yellow color is proportional to the human iL8 concentrations in samples.

2.3. Statistical Analysis

Statistical analysis of data was conducted using SPSS 11.5 for windows software. Differences between observations were considered significant at P≤ 0.01, P≤ 0.05. The following statistical methods were used for the analysis of data:

1. Standard statistical methods were used to determine the number, percentage, the mean, standard deviation (SD) and standard error.
2. Paired student t-test was used among the factors for comparisons.
3. Pearson correlation coefficient was used to find the relationship among the measured variables.

3. Results

The distribution of RAU in the oral cavity of RAU cases is shown in Figure 1. The highest number of RAU was in the lower lip (30%) followed by buccal mucosa (26%) and the lowest was seen on the dorsum of the tongue (4%).

The levels of iL-8 in both RAU and control groups was estimated and showed that the mean serum level of iL-8 in RAU cases was 404 pg/ml compared to 305 pg/ml in control group and the difference was highly significant P≤0.01 (Figure 2).
Concerning different age groups in RAU patients, the level of IL-8 was higher in age group 13-30 years than other age groups and the difference was not significant (Figure 3).

According to gender, female with RAU showed higher level of IL-8 compared to male and the difference was not significant (Figure 4).

Two types of RAU, Major and minor, the level of IL-8 was higher in minor compared to major and the difference was not significant (Figure 5).
4. Discussion

The study aimed to estimate the concentration of IL-8 in RAU and control groups. The present study showed high level of IL-8 in RAU compared to control group. Earlier studies showed the presence of different immune cells in RAU lesion that produce different cytokines.

Cytokines play an important role in numerous biological activities including proliferation, development, homeostasis, regeneration, repair, and inflammation [11]. Earlier studies have shown the presence of T and B lymphocytes, mature tissue macrophages, monocytes in oral aphthous lesion [12]. Increased production of TNF-α by peripheral blood leukocytes are found in active RAU patients. This locally and systemically produced TNF-α can induce many cell types to secrete IL-8 in the local tissue and peripheral blood [13].

In the present study, the level of IL-8 was significantly higher in RAU patients compared to controls. Sun et al [14] showed an increased plasma level of IL-2 in patients with active stage of RAU, while other study showed significant increase in serum levels of IL-8 and IL-6 in patients compared to controls [15,16,17]. Keratinocytes, endothelial cells and neutrophils in pre-ulcerative oral aphthous lesion secrete significant amount of IL-8 which activates neutrophils and attract more T cells, including cytotoxic T cells to the aphthous lesion. IL-8 activates neutrophils, which produces enzymes causing tissue destruction. Thus IL-8 may be involved in the pathogenesis of RAU [8].

In this study, females with RAU showed higher level of IL-8 compared to male and the difference was not significant. In other study all patients showed significant increase in plasma levels of progesterone, IL-8 and IL-10. Plasma levels of IL-10 were significantly higher in female patients [18]. Another study showed that the levels of the pro-inflammatory cytokines, IL-6 and IL-8, were increased in male and in female cells [19]. In relation to the female predisposition to RAU, some authors have suggested that this association is related to hormonal rates [20].

In present study, two types of RAU were diagnosed, major and minor, the level of IL-8 was higher in minor compared to major and the difference was not significant. Devi et al, [21] found that minor RAU was the most common variant, constituting 80% of RAU while major RAU affected about 10–15% of patients. Ulcers vary from 8 to 10 mm in size and are most commonly seen on the non-keratinized mucosal surfaces. The current study showed that most RAU were located on the lower lips followed by buccal mucosa.

The level of IL-8 showed increased in age group 13-30 years old. There is some evidence that the RAU has a higher prevalence in younger adults, decreasing in both incidence and severity with age [22,23].

5. Conclusion

High concentration of IL-8 was noticed in RAU patients compared to control group. Age group 13-30 years old as well as females showed high concentration of IL-8.

Statement of Competing Interests

The authors declare that they have no conflicts of interest.

References

[1] Scully C, and Porter S. Oral Mucosal Disease: Recurrent Aphthous. Brit J Oral Maxill Surg. 46(3): 198-206. 2008.
[2] Axell T and Henriksson V. The Occurrence of Recurrent Aphthous Ulcers in an adult Swedish Population. Act Odontol Scand. 43: 121-125. 1985.
[3] Saraceno R1, Perugia C, Ventura A, LORè B, Chimenti S, Dociamo R., Aphthous, celiac disease and other dental disorders in childhood. G Ital Dermatol Venerol. 151(3): 239-243. 2016.
[4] Belenguer-Guallar I, Jiménez-Soriano Y, and Clarumunt-Lozano A. Treatment of recurrent aphthous stomatitis: A literature review. J Clin Exp Dent. 6(2): 168-174. 2014.
[5] Sbibiola Z, Szoñor E, Kowalska A. Etiopathogenesis of recurrent aphthous stomatitis and the role of immunologic aspects: literature review. Arch Immunol Ther Exp (Warsz). 62(2): 205-215. 2014.
[6] Buno IU, Huff JC, Weston WL. Elevated Levels of Interferon Gamma, Tumor Necrosis Factor Alpha, Interleukins 2, 4, and 5, but not Interleukin 10, are present in Recurrent Aphthous Stomatitis. Arch. Dermatol. 134(7): 827-831. 1998.
[7] Sun A, Chang YF, Chia JS, Chiang CP. Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of recurrent aphthous ulcers. J Oral Pathol Med. 33: 133-139. 2004.
[8] Katsantonis J, Adler Y, OrfanoS CE, Zouboulis CC. Adamantnides-Beheçêt's disease: Serum IL-8 is a more reliable marker for disease activity than C-reactive protein and erythrocyte sedimentation rate. Dermatol. 201: 37-39. 2000.
[9] Brozovic S, Vucicicicic S, Boras V, and Bukovic D. Serum IgA, IgG, IgM and salivary IgA in recurrent aphthous ulceration. Coll Anthropol. 25(2): 633-637. 2001.
[10] Martinez OK, Mendes LL, Alves BJ. Secretary A immunoglobulin, total proteins and salivary flow in Recurrent Aphthous Ulceration. Rev Bras Otorrinol. 73(3): 323-328. 2007.
[11] Okada H, and Murakami S. Cytokine expression in periodontal health and disease. Crit Rev Oral Biol Med. 9: 248-266. 1998.
[12] Sun A, Wang JT, Chia JS, Chiang CP. Levamisole can modulate the serum tumor necrosis factor-alpha level in patients with recurrent aphthous ulcerations. J Oral Pathol Med. 35: 111-116. 2006.
[13] Al-Dalaan A, al-Sedairy S, al-Balaa S, al-Janadi M, Elramahi K, Bahabri S et al. Enhanced interleukin 8 secretion in circulation of patients with Behçet's disease. J Rheumatol. 22: 904-907. 1995.
[14] Sun A, Chu CT, Liu BY, Wang JT, Lee JS and Chiang CP. Expression of Interleukin-2 Receptor by Activated Peripheral Blood Lymphocytes Upregulated by the Plasma Level of Interleukin-2 in Patients with Recurrent Aphthous Ulcers. Proc. Natl. Sci. Counc. Repub. China B 24(3): 116-122. 2000.
[15] Bakhtiari B. Vaziri PB, Hajilooi M, Mortazavi H. Evaluation of serum levels of interleukin-6 and interleukin-8 in patients with recurrent aphthous ulcerations. Rev of Glob Med Healthcare Res. 3(1): 410. 2010.
[16] Gür-Toy G, Lenk N, Yalcin B, Aksaray S, Alii N. Serum interleukin-8 as a serologic marker of activity in Behçet's disease. Int J Dermatol. 44(8): 657-660. 2005.
[17] Sun A, Chang YF, Chia JS, Chiang CP. Serum interleukin-8 level is a more sensitive marker than serum interleukin-6 level in monitoring the disease activity of recurrent aphthous ulcerations. J Oral Pathol Med 33: 133-139. 2004.
[18] Trotter A, Mück K, Grill H,Schirmer UW, Hannekum A, Lang D. Gender-related plasma levels of progesterone, interleukin-8 and interleukin-10 during and after cardiopulmonary bypass in infants and children. Critical Care. 5(6): 343-348. 2002.
[19] DaPozzo E, Giaconelli C, Cavallini C, and Claudia Martini C. Cytokine secretion responsiveness of lymphomonocytes following cortisol cell exposure: Sex differences. PLoS One. 13(7): e0200924. 2018.
[20] Ship JA, Chavez EM, Doerr PA, Henson BS, Sarmadi M. Recurrent aphthous stomatitis. Quintessence Int. 31: 95-112. 2000.

[21] M K PD, D N S V R, Koppal S, Byatnal AR, Rukmangada T, Byatnal AA. Efficacy of Rebamipide and Levamisole in the Treatment of Patients with Recurrent Aphthous Ulcer - A Comparative Study. J Clin Diagn Res. 8(11): ZC119-ZC122. 2014.

[22] McRobbie H, Hajek P, Gillison F. The relationship between smoking cessation and mouth ulcers. Nicotine Tob Res. 6: 655-665. 2004.

[23] Abdullah MJ. Prevalence of recurrent aphthous ulceration experience in patients attending Pyramid dental specialty in Sulaimani City. J Clin Exp Dent. 5(2): e89-e94. 2013.

© The Author(s) 2021. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).