

Establishment of Role of IL-2, IL-10 and IL-12 in Patients with Recurrent Aphthous Stomatitis—A Clinical Study

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ABSTRACT

Background: Recurrent aphthous stomatitis is a vesiculobullous disease characterized by painful ulcers in the oral cavity. The role of interleukins such as IL-2, IL-10 and IL-12 in initiating disease demands careful assessment. The present study was conducted to determine the level of IL-2, IL-10 and IL-12 in patients with recurrent aphthous stomatitis.

Materials and methods: The present study was conducted on 40 patients diagnosed with recurrent aphthous stomatitis. An equal number of age and gender-matched subjects (40) was included as a control. They were divided into 2 age groups from 20 to 40 years and 40 to 60 years. All were made to sit comfortably on a dental chair, and 1 ml of unstimulated saliva was collected in a sterile tube to assess the level of IL-2, IL-10 and IL-12 using ELISA. The level of IL-2, IL-10 and IL-12 was measured in pg/mL.

Results: Each group had 10 males and 10 females. The difference was non-significant (P=1). Age group 20-40 years comprised of 14 patients in group I (eight males and six females) and 12 in group II (five males and seven females). Age group 40-60 years had six patients in group I (two males and four females) and 8 patients in group II (five males and three females). The difference was significant (p < 0.05). The most common form was minor (82%) followed by herpetiform (13%) and major (5%).

In group I, the mean value of IL-2 level was 32.24 pg/mL, IL-10 was 1.24± 0.6 and IL-12 was 28.34± 4.04 and in group II, mean value of IL-2 level was 12.10 pg/mL, IL-10 was 2.56± 1.12 and IL-12 was 23.16± 4.16. The difference was significant (p < 0.05).

Conclusion: Age group 20 to 40 years showed higher prevalence. The level of IL-2 and IL-12 is highly increased while IL-10 is decreased in patients.

Clinical significance: Role of interleukins as a precipitating factor along with stress is well established. With the thorough knowledge of the disease process, the newer treatment modality specific against interleukins may be proven useful in controlling the disease.

Keywords: Interleukins, Recurrent aphthous stomatitis, Stress

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INTRODUCTION

Recurrent aphthous stomatitis is a vesiculobullous disease characterized by painful ulcers in the oral cavity. It is of three types, aphthous major, aphthous minor and herpetiform ulcers. A fourth form is also seen associated with Behcet's disease. It appears on non-keratinized mucosa such as buccal mucosa, ventral surface of the tongue, soft palate and attached gingival. It is of round or ovoid shaped with yellowish necrotic slough surrounded by erythematous halos. As it appears again and again at irregular intervals, it is known as recurrent aphthous stomatitis. It has been observed that 25 to 30% of the population suffer from RAS.¹

Recurrent aphthous major is of considerable size lasts up to 6 weeks. Recurrent aphthous minor occurs in clusters are small than major (usually 5mm) lasts for

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10 to 14 days and herpetiform ulcers appears in dozens (up to 100) similar to herpetic ulcers. All heals by itself without scar except recurrent aphthous major. The exact etiology is still unknown.

Predisposing factors of RAS include genetic predisposition, nutritional deficiency, hematologic abnormalities, anger, and hormonal changes, etc. Role of stress and trauma is well documented.² It is cell-mediated immune response characterized by the production of interleukins such as IL-2, IL-10, IL-12, TNF-alpha and IFN delta, etc. These interleukins along with IL-2 induces a cell-mediated immune response. There is increased expression of IL-2 and IL-12 in patients with recurrent aphthous stomatitis.³ The present study was conducted to determine the level of IL-2, IL-10 and IL-12 in patients with recurrent aphthous stomatitis.

MATERIALS AND METHODS

The present study was conducted in the Department of Oral Pathology and Microbiology. It comprised of 40 patients of both genders with the complaint of recurrent aphthous stomatitis. The equal number of age and gender-matched subjects (40) was included as a control in the study. All were informed regarding the study, and written consent was obtained. Ethical clearance was taken from institutional ethical committee prior to the study.

General information such as name, age, gender, etc. was recorded in cases history perform. Subjects in the age range of 20 to 60 years were considered. Patients with the history of systemic diseases, anemia or vitamin deficiency, smokers, and periodontal diseases were excluded from the study. Selection of cases was done after obtaining a history and thorough clinical examination.

They were divided into two age groups from 20 to 40 years and 40 to 60 years. All were made to sit comfortably on a dental chair and allowed to rinse their mouth with water. They were asked to expectorate 1 mL of unstimulated saliva in a sterile tube. Saliva sample collected from each subject was stored at 80 degree Celsius and subjected to ELISA.

For the estimation of IL-2, IL-10 and IL-12, microtitre plate was pre-coated with the antibody against interleukins. Saliva samples were added to the microtiter plate well with biotin-conjugated polyclonal antibody preparation followed by addition of Avidin conjugated to HRP to each well and incubation was done. TMB substrate was added to this. There was color change in wells containing IL-2, 10 and 12. ELISA microplate reader performed the measurement of color at 450 nm wavelength. The level of IL-2, IL-10 and IL-12 was measured in pg/mL. Results thus obtained were analyzed using 14.00 SPSS. Mean+ S.D was obtained

and independent t-test was used to obtain a level of significance. The p-value less than 0.05 was considered significant.

RESULTS

Patients were divided in group I and controls were put in group II. Each group had 10 males and 10 females. The difference was non-significant (P=1) (Table 1). Table 2 shows that age group 20 to 40 years had 14 patients in group I and 12 in group II, 40 to 60 years had 6 in group I and 8 in group II. The difference was significant ($p < 0.05$). Out of 20 patients in group I, 20 to 40 years had 8 males and 6 females, 40 to 60 years had 2 males and 4 females (Graph 1). The difference between age groups was significant ($P < 0.05$). Graph 2 shows that out of 20 patients in group II, 20 to 40 years had 5 males and 7 females, 40 to 60 years had 5 males and 3 females. The difference was significant ($p < 0.05$).

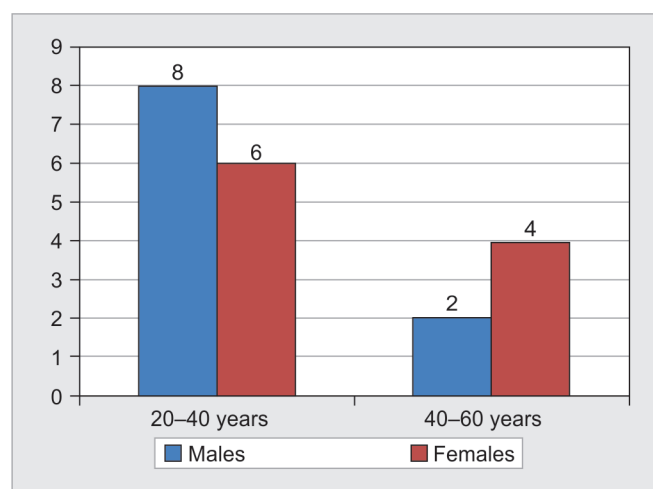
Table 1: Distribution of participants

Total-40				p-value
Group I (cases)		Group II (control)		
Males	Females	Males	Females	1
10	10	10	10	

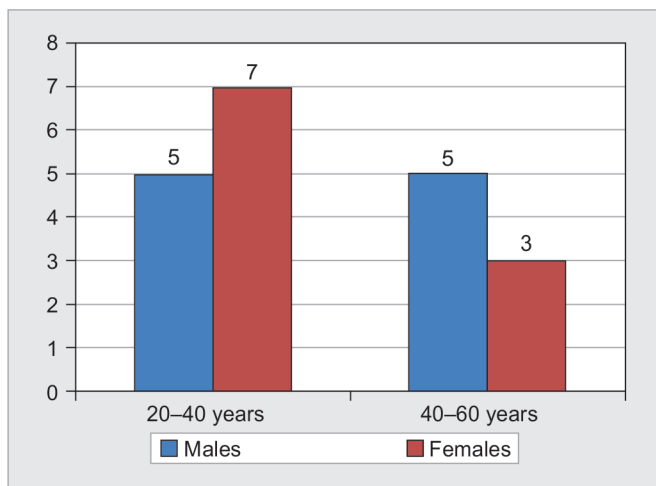
Table 2: Age wise distribution of participants

Age group	Group I	Group II	Total	p-value
20 to 40 years	14	12	26	0.01
40 to 60 years	6	8	14	
Total	20	20	40	

The most common form was minor (82%) followed by herpetiform (13%) and major (5%) (Table 3). In group I, the mean value of IL-2 level it was 32.24 pg/mL with S.D 3.58, and in group, II was 12.10 pg/mL with S.D 1.24. t value found to be 24.01 and the difference in IL-2 level in both groups was highly significant ($p=0.001$). While comparing IL-10 level, the mean value was 1.24 ± 0.6 in



Graph 1: Gender and age wise distribution in group I



Graph 2: Gender and age wise distribution in group II

group I and 2.56 ± 1.12 in group II. The difference was significant ($P < 0.05$). IL-12 was 28.34 ± 4.04 in group I and 23.16 ± 4.16 in group II, the difference was statistically significant ($p = 0.01$) (Table 4).

Table 3: Clinical types of RAS

Types	Percentage	p-value
Minor	82	
Major	5	0.01
Herpetiform	13	

Table 4: Estimation of IL-2 level in both groups

Groups	Mean \pm S.D		T value	p-value
	Group I	Group II		
IL-2	32.24 ± 3.58	12.10 ± 1.24	24.01	0.001
IL-10	1.24 ± 0.6	2.56 ± 1.12	6.35	0.05
IL-12	28.34 ± 4.04	23.16 ± 4.16	17.43	0.01

DISCUSSION

Recurrent aphthous stomatitis is a well known oral mucosal lesion characterized by multiple painful ulcers in an oral cavity. The pathogenesis involves stimulation of keratinocytes leading to activation of interleukins such as IL-2, IL-10 and IL-12. Studies have shown that in the case of RAS, there is enhanced lipid peroxidation and increase in oxidative stress in the blood.⁴ There is a suppressed anti-oxidative process in the blood. It has been found that during the process, there is a significant increase in the IL-2 and IL-12 in the blood. In the mean time, the level of IL-10 is decreased. The function of IL-10 is to inhibit the production of cytokines which initiates the aphthous ulceration in the mouth. With the increase in cytokines in the blood, there is a reduction in the suppression of inflammatory mechanism which in turns results into severe ulceration.⁵ The present study was aimed to determine the role of interleukins such as IL-2, IL-10 and IL-12 in recurrent aphthous stomatitis.

In the present study, we included 20 patients in group I who had RAS and sex and age-matched 20 subjects as controls in group II. Both groups had equal numbers of males (10) and females (10). Nowak et al.⁶ conducted a study on patients with RAS and normal subjects. The comparison of IL-2 concentration in peripheral blood and stimulated saliva was performed in both groups. Authors found significant increases in IL-2 level in patients with RAS in blood, but there was no alteration in IL-2 level in saliva.

In this study, we found that a maximum number of patients was seen in age groups 20 to 40 years. There were 26 patients, 14 in group I and 12 in group II. In group I, there were eight males and six females while in group II, there were five males and seven females. Age group 40 to 60 years comprised of 14 patients, 6 in group I and 8 in group II. We found two males and four females in group I and five males and three females in group II. Amador-Pattaroyo et al.⁷ found that RAS is the disease of childhood and peak was observed in second decades. In the present study, we found that in 82% of cases minor type was seen followed by herpetiform (13%) and major (5%). Interleukin level is independent of types of RAS. Gorska⁸ conducted a study of oral mucosa changes and found that RAS was commonly encountered in children, adolescents and adults between 13–24 years of age.

In our study, we measured the salivary level of IL-2, IL-10 and IL-12 in both groups using ELISA. We observed that IL-2 was highly increased in RAS group (group I) as compared to group II. Our results are in accordance to the study by Albanidou-Farmaki et al.⁹ who found similar results. There was the enhanced production of t-lymphocytes which elevates the level of IL-2 and IL-12. In addition the level of IL-10 was decreased in group I. Buno et al.¹⁰ conducted a study in which the level of interferon gamma, tumor necrosis factor alpha, and interleukins 2, 4, 5 and 10 was measured. Authors found the elevation of interleukins 2, 4 and 5 whereas IL-10 was significantly fallen in recurrent aphthous stomatitis patients.

Similarly, in the present study, the level of IL-10 was lowered in group I although the difference was non-significant ($p > 0.05$). IL-12 was also raised in group I as compared to group II. Miyamoto et al.¹¹ in their study of immune expression of HSP27 and IL-10 in recurrent aphthous ulceration found that IL-10 was reduced in RAS group.

Lewkowicz et al.¹² found that the level of IL-10 is greatly decreased in patients with RAS. This potentiates the finding that the imbalance in interleukin level may stimulate the process of RAS in patients. Guimaraes et al.¹³ investigated gene polymorphisms of IL-1beta, IL-6, IL-10 and TNF-alpha in individuals with recurrent

aphthous stomatitis and found that IL-10 is significantly decreased which is a useful anti-inflammatory agent.

TNF- α is a pro-inflammatory cytokine which causes activation of neutrophils and cytotoxic T-cells and leads to the production of ulceration in the mouth. The role of IL-2 in disease process can be judged by the fact that their level is significantly enhanced in patients of RAS.¹⁴ Keenan et al.¹⁵ along with IL-2 level elevation in patients also suggested the role of stress in the development of a disease. The author concluded that in stressful condition, the immune system is altered and there is a cascade leading to stimulation of T-lymphocytes which in turn releases IL-2 and IL-12. Hence stress is a potential risk factor for the disease.

Natah et al.¹⁶ in their study of immunolocalization of tumor necrosis factor alpha-expressing cells in recurrent aphthous ulcer lesions suggested the role of TNF-alpha and IL-2 in the pathogenesis of severe ulceration in patients.

CONCLUSION

Recurrent aphthous stomatitis is a common, occurring mucosal disease characterized by ulceration in the oral cavity. The level of IL-2 and IL-12 is highly increased while IL-10 is decreased in patients. Age group 20 to 40 years showed higher prevalence.

CLINICAL SIGNIFICANCE

Role of interleukins as a precipitating factor along with stress is well established. With the thorough knowledge of the disease process, the newer treatment modality specific against interleukins may be proven useful in controlling the disease.

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