

How to Cite:

Marsoul, R. D., Al- Qaysi, S. A., & Alsaady, M. (2022). Investigation of tumour necrosis factor-alpha role in development of recurrent aphthous stomatitis. *International Journal of Health Sciences*, 6(S1), 218-222. <https://doi.org/10.53730/ijhs.v6nS1.4748>

Investigation of Tumour Necrosis Factor-Alpha Role in Development of Recurrent Aphthous Stomatitis

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Abstract---Recurrent aphthous stomatitis (RAS) is a common disease characterized by a repeated formation of non-contagious and benign oral ulcers. The cause for this condition not completely understood but it involves a T cell-mediated immune response initiated and triggered by different factors. The understanding of the pathogenesis of the disease is essential to improve the plan of management. Objective: This study aimed to identify the correlation between the blood levels of tumour necrosis factor-alpha (TNF- α) levels and the development of RAS. Subjects and Methods: Present study involved 100 participants of which 50 subjects diagnosed with RAS and the others without (healthy controls). Blood samples obtained from participants and used for TNF- α investigation by enzyme linked immunosorbent assay. The data collected was statistically analysed. Results: Serum TNF- α concentrations were significantly ($P < 0.05$) higher in patients with RAS (mean \pm standard deviation [SD]; 104.27 \pm 41.63) compared to control group (mean \pm SD; 85.30 \pm 38.72). Conclusions: Serum TNF- α is increased in Iraqi patients with RAS compared to controls. RAS is one of the significant variables that relates to high TNF- α serum concentration.

Keywords---aphthous stomatitis, common disease, investigation, recurrent ulcers, tumour necrosis.

Introduction

Aphthous stomatitis is a common disease characterized by the repeated formation of non-contagious and benign oral ulcers called (aphthae) in otherwise healthy persons. The term (canker sores) used mainly in North America, although this may also refer to any ulcers inside the mouth. The cause for this condition is not completely understood but it involves a T cell-mediated immune response initiated and triggered by different factors. These factors may include local trauma, nutritional deficiencies stress, hormonal influences, certain foods, allergies, dehydration, genetic predisposition or certain food additives.

These sores occur periodically and completely heal between the attacks. In the majority of cases, the individual ulcers persist about (7–10) days, and the ulceration episodes occur (3–6) times per one year. Most of these ulcers appear on the non-keratinizing epithelial surfaces in the oral cavity – i.e. anywhere except in the attached gingiva, the dorsum of the tongue and the hard palate – although the more severe forms that are less common might also involve keratinized epithelial surfaces. Symptoms range from a minor nuisance to the interfering with the drinking and eating. The severe forms might be debilitating and even causing weight loss due to malnutrition.

This disease is very common; it is affecting about (20%) of the general population to some degree (Bruch & Treister, 2010; Namrata & Abilasha, 2017). The onset is often during adolescence or during childhood; and the condition usually remains for several years before gradually disappearing. There is no curing and the treatments like corticosteroids aim for managing pain, reducing of the healing time and reducing of the frequency of ulceration episodes. TNF- α is a pro-inflammatory factor and is one of the most important cytokines that implicated in the presence and development of new aphthous ulcers in the oral cavity.

The association of this cytokine in the development of RAS gains confidence due to fact that immunomodulatory drugs like pentoxifylline and thalidomide had been found effective in the RAS treatment. Pentoxifylline inhibits the production of TNF- α and thalidomide reduces activity of TNF- α by degrading its messenger RNA (Jacobson et al., 1997; Zabel et al., 1993). Antigenic stimulation of keratinocytes in the oral mucosa leads to the production of pro-inflammatory cytokines like TNF- α and IL-2. TNF- α also results in the expression of class I major histocompatibility complex, and subsequently these cells targeted for attack by cytotoxic T cells (Bruch & Treister, 2010).

Materials and Methods

Study design

The present study includes 100 participants. These participants were divided into two groups, the first group includes 50 RAS patients and the second group includes 50 apparently healthy individuals (control group). The current study was carried out from the outpatient clinic (dental health centres and dental department in Al-Husain Medical City/ Holy Karbala province, Iraq), and the samples were under the supervision of specialist dentists.

This study was performed in the laboratories of the Biochemistry Department in Faculty of the Medicine/ University of Babylon. In total, 50 patients (48 % of females and 52 % of males) were enrolled in the study with RAS based on the history and clinical features for RAS. Body Mass Index (BMI) was calculated by weight (kg) divided by the square of height (m), $BMI = \text{Weight (kg)} / \text{Square Height (m}^2\text{)}$. Exclusion criteria were the presence of chronic systemic diseases such as cardiovascular diseases, hypertension and hormonal disturbances, identified by a physician, and the presence of other seriously interfering diseases, subjects < 6 years old and non-agreement.

Sample collection

Two milliliters of venous blood was taken from patients and drained into gel plain tube for serum preparation, which would be used in TNF- α test. Blood in gel tube allowed to clot for 40 min at 37°C and then centrifuged at 3000xg for approximately 10 min. The serum has been stored at -20°C until the time of assay. The concentrations of serum TNF- α were determined at laboratories of Biochemistry Department, Faculty of Medicine, University of Babylon by enzyme-linked immunosorbent assay (ELISA) using human TNF- α ELISA kit (PARS BIOCHEM) (Takeshita et al., 1993).

Data analysis

The data analyzed using the Software Package for Social Sciences version 21 (SPSS, IBM Company, Chicago, USA). The data were presented as a mean \pm standard deviation (SD), the differences between studied groups were evaluated using unpaired Student's *t*-test. The associations between TNF- α blood levels were tested using Pearson's correlation coefficient.

Results

There was no significant difference in age and body mass index (BMI) (as mean) between control and RAS group ($P = 0.97$ and 0.61), as shown in table 1. This age and BMI matching helps to eliminate differences in parameter results that may originate due to the significant variation in age and BMI. Serum TNF- α concentrations were significantly ($P < 0.005$) higher in RAS patients (mean \pm SD; 104.27 ± 41.63) than that of control group (mean \pm SD; 85.30 ± 38.72) as illustrated in table 2.

Table 1
Anthropometric parameters in the studied groups

			Groups		Total	P-value
			Patients	Controls		
Age	≤ 20	N	7	6	13	0.97
		%	14.0%	12.0%	13.0%	
	21-30	N	14	16	30	
		%	28.0%	32.0%	30.0%	
	31-40	N	18	17	35	
		%	36.0%	34.0%	35.0%	

BMI	41-50	N	11	11	22	0.61
		%	22.0%	22.0%	22.0%	
	Normal	N	4	7	11	
		%	8.0%	14.0%	11.0%	
	Overweight	N	14	12	26	
		%	28.0%	24.0%	26.0%	
	Obesity	N	32	31	63	
		%	64.0%	62.0%	63.0%	

Table 2
Mean difference TNF- α between RAS patients and controls

	Group	No.	Mean \pm SD	P-value
TNF A	P	50	104.27 \pm 41.63	< 0.05
	C	50	85.30 \pm 38.72	

Discussion

RAS is the most common inflammatory disease of the oral mucosa with a global prevalence of 0.5% to 75% and female predilection (Liang & Neoh, 2012). The first episode of RAS most frequently commences in the second decade of life. The lesions usually begin with prodromal burning sensation 2 to 48 hours before an ulcer appears (Greenberg et al., 2000). Oral aphthous ulcers typically occur as painful, symmetrically round fibrin-covered mucosal defects with an erythematous border and most commonly on non-keratinized mucosa in healthy patients. However, it can be seen on the keratinized mucosa especially in patients with immune deficiency (Mortazavi et al., 2016). It was demonstrated that there was a significant ($P < 0.05$) increase in the level of TNF in patients in comparison with the control (104.27 \pm 41.63 vs. 85.30 \pm 38.72) (Shen et al., 2021).

Tumour necrosis factor alpha (TNF- α) is a pro-inflammatory factor and is one of the most important cytokines implied in the development of new aphthous ulcers in patients. The association of TNF- α in the development of RAS gains credence due to the fact that immunomodulatory drugs such as thalidomide and pentoxifylline have been found effective in the treatment of RAS. Thalidomide reduces activity of TNF- α by degrading its messenger RNA and pentoxifylline inhibits TNF- α production (Preeti et al., 2011). Antigenic stimulation of oral mucosal keratinocytes results in the production of pro-inflammatory cytokines such as IL-2 and TNF- α . TNF- α also causes expression of class I major histocompatibility complex, subsequently these cells are targeted for attack by cytotoxic T cells (Jurge et al., 2006). Cytokines have a key role in the pathogenesis of certain diseases and were considered as primary factors that initiate the cytotoxicity (Gulati et al., 2016).

The levels of salivary tumour necrosis factor-alpha in recurrent aphthous stomatitis patients were no significantly increased. Considering that serum TNF- α was mostly within the normal range, its role in the pathology of RAS needed to be further explored (Chaudhuri et al., 2018). Some studies indicates that TNF- α has important effects on the RAS development. Numerous reports suggest that factors such as stress, hematinic deficiency, trauma, genetics, and cytokines can be

effective in the formation of RAS. TNF- α is a main pro-inflammatory cytokine that plays an important role in immune and inflammatory responses (Greenberg et al., 2000). TNF- α actually shows important immunomodulatory activities and studies have shown its relationship with RAS. Thus, high levels of TNF- α had been reported in wound mucosa and peripheral blood of patients with aphthous ulcer (Mortazavi et al., 2016). High cytotoxic destruction of epithelial cells with TNF- α produced from peripheral blood mononuclear cells was shown in patients with aphthous ulcer. In addition, RAS can be prevented by inhibitors of endogenous TNF- α synthesis such as thalidomide and pentoxifylline (Shen et al., 2021). Certain study showed that level of tumour necrosis factor-alpha in recurrent aphthous stomatitis patients were significantly increased. Considering that serum TNF- α was mostly within the normal range, its role in the pathology of RAS needed to be further explored (Preeti et al., 2011).

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