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Serum IgA, IgG, IgM and Salivary IgA in Recurrent Aphthous Ulceration

S. Brozović[1], V. Vučićević-Boras[1] and D. Buković[2]

[1] Department of Oral Medicine, School of Dental Medicine, Zagreb, Croatia
[2] Department of Prosthodontics, School of Dental Medicine, Zagreb, Croatia

A B S T R A C T

Radial immunodiffusion technique was used to estimate salivary immunoglobulin A, and enzyme-linked immunosorbent assay for estimation of serum IgA, IgG and IgM in 30 patients with acute recurrent aphthous ulceration (RAU) and during remission period compared to 30 healthy controls. Significantly elevated level of salivary IgA ($p < 0.05$) was found in patients with minor RAU when compared to the control group. Serum IgA level was elevated in patients with minor acute RAU when compared to the controls ($p < 0.05$). Serum immunoglobulin level of IgG and IgM showed no differences between patients with either minor or major recurrent aphthous ulceration and controls.

Introduction

Recurrent aphthous ulceration (RAU) is a common oral disease appearing usually on nonkeratinized oral mucosa, especially on the tongue, vestibulum, palate and buccal mucosa[1]. The etiology of the disease is still not completely understood. Many local and systemic factors have been associated with these conditions as well as evidence of a genetic and immunopathogenic basis for RAU[2,3]. Evidence suggests that RAU is connected with chronic bowel disease, haematinic deficiencies, AIDS, food hypersensitivity, radiation and severe stress[4,5]. Some authors[6] suggested the possible viral etiology in the development of RAU. The common prevalence is 20% and up to 55% depending on population sampling and criteria used for inclusion in this symptom complex[7,8]. The most common presentation are minor recurrent aphthous ulcers: round, painful ulcers up to 10 mm in diameter that heal within 10–14 days without scarring. Major ulcers are larger than 10 mm in diameter, and can last for several weeks and frequently scar. Numerous authors have investigated cellular and humoral immune response of patients with RAU[9–11]. A study by Sistig et al.[12] showed alterations in cellular immu-

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nity especially in phagocytosis, ADCC, and NK cells activity. Various authors have reported that patients with RAU show changes in the serum immunoglobulin levels[13,14].

The aim of our study was to examine the involvement of humoral immunity in the development of RAU and to determine the role of salivary IgA in the process of healing in patients with RAU.

Material and Methods

The study population consisted of 30 patients (16 men and 14 women), 22 with minor ulcers and 8 with major ulcers (age range 17–79 yrs, mean age 40 yrs) with clinical features, history and verified diagnosis of RAU. Informed consent according to Helsinki II was obtained from each participant. Criteria for differentiation of the aphthae were diameter of the lesion, according to Lehner[15]. Those less than 10 mm in diameter were considered as minor, while those bigger than 10 mm as major. We analyzed patients with acute RAU (ulcers 2 days old) as well as during remission period (1 week after healing). None of the patients were smokers, nor had received previous treatment for RAU. Thirty healthy volunteers (18 men and 12 women) with no history of RAU served as controls, they all had healthy oral mucosa (age range 20–58 yrs, mean age 39 yrs). Peripheral blood samples (5ml) were taken by venepuncture from patients and controls, between 8–12 a.m. into plastic tubes. Serum was centrifuged and aliquots were stored at –20 °C until ready to be used. Participants were asked to sustain from eating and drinking for two hours prior to saliva collection. The unstimulated whole saliva was collected between 10–12 a.m. according to Wu-Wang et al.[16]. All participants were instructed to collect saliva in their mouths for 5 min without swallowing and to spit into a clean plastic container. Salivary flow rate was recorded. The pooled samples were immediately placed in a –20° freezer until ready for analysis.

The level of IgA in saliva was determined by radial immunodiffusion technique according to Mancini at al.[17] using specific anti-human 11S IgA serum (Institute of Immunology, Zagreb, Croatia). During the determination the samples of saliva were diluted three times. In determined places, standards of 11S IgA and diluted samples were applied. The process of immunodiffusion was continued for 48 hours at room temperature. In order to remove unbound proteins the plates were washed 48 hours in 3% (w/v) sodium chloride with several changes of washing solution. The excess amount of sodium chloride was removed by washing the plates with distilled water during the next 24 hours. To ensure visibility of the bands obtained by immunoprecipitation, the plates were placed for 45 minutes in 1% (w/v) solution of tannic acid and then washed with distilled water. The diameter of the precipitating ring was measured by radial immunodiffusion reader. The concentration of tested samples was estimated by using standard calibration curve, and expressed in mg/ml.

Serum IgA, IgG and IgM were determined using commercially available double sandwich ELISA technique (Northeast Biomedical Laboratories, Uxbridge, Middlesex).

Statistical analysis was performed using ANOVA test, and values lower than 0.05 were considered as significant.

Results

Serum IgA values were significantly different between patients with acute minor RAU and controls ($p < 0.05$; $F=1.23$) (Table 1). Serum IgA values in patients with major RAU were not significantly different in acute RAU, during remission compared to controls. Serum IgG and IgM values did not differ significantly between patients with minor and major

acute RAU, during remission period, and controls. Salivary IgA values differed significantly during the acute stage of recurrent aphthous ulcers in patients with minor RAU, when compared to controls ($p < 0.05$; $F=6.85$). The values of salivary IgA were significantly higher in the acute stage of the disease than in controls. In major RAU there was no difference in salivary IgA between acute stage, remission and healthy controls (Table 1).

Salivary flow rates did not differ significantly between patients with acute RAU (either minor or major), during remission period and controls (Table 2).

TABLE 1
SERUM IMMUNOGLOBULINS A, G, M AND SALIVARY IMMUNOGLOBULIN A LEVELS IN ACUTE PHASE AND DURING REMISSION PERIOD IN PATIENTS WITH MINOR AND MAJOR RECURRENT APHTHOUS ULCERS AND IN CONTROLS, AS WELL AS IN STUDY OF PORTER ET AL.[8]

	Acute		Remission		Control	
	(X	SD)	(X	SD)	(X	SD)
Minor RAU						
IgA (mg/mL)	3.143	0.721	2.732	0.833	2.764	2.482
IgG (mg/mL)	11.936	2.856	12.956	2.513	12.059	3.259
IgM (mg/mL)	1.248	0.717	1.439	0.772	1.258	0.422
sIgA (mg/mL)	0.079	0.026	0.065	0.002	0.054	0.024
MAJOR RAU						
IgA (mg/mL)	2.728	1.162	2.534	1.183	2.764	2.482
IgG (mg/mL)	11.977	2.743	13.217	2.605	12.059	3.259
IgM (mg/mL)	1.138	0.510	1.341	0.548	1.258	0.422
sIgA (mg/mL)	0.068	0.021	0.055	0.024	0.054	0.024
Porter et al.	Number of patients with RAU					
IgA (mg/mL)	(1)					
IgG (mg/mL)	(1)					
IgM (mg/mL)	(5)					
IgM, IgG (mg/ml)	(1)					

TABLE 2
COMPARISON OF SALIVARY FLOW RATES IN PATIENTS WITH MINOR AND MAJOR RAU DURING ACUTE PHASE AND REMISSION PERIOD AND IN CONTROLS, AS WELL AS IN STUDY OF WU-WANG ET AL.[16]

Major RAU (ml/min)			Minor RAU (ml/min)			Controls (ml/min)	
Acute	0.54	0.07	Acute	0.55	0.05	0.59	0.06
Remission	0.56	0.05	Remission	0.57	0.08		
Wu-Wang et al.							
Acute	0.55	0.04				0.58	0.07
Remission	0.52	0.07					

Discussion

Salivary immunoglobulin A in patients with recurrent aphthous ulceration has not yet been investigated comprehensively. Many studies[18–20] have been performed with the aim of investigating the physiology of salivary IgA and its connection with diseases of the oral mucosa such as recurrent aphthous ulcerations.

The results of our study show that serum IgA levels are only elevated in minor acute RAU when compared with controls. In patients with major acute RAU, and during remission period, levels of serum IgA remained within the physiologic range. Serum IgG and IgM levels did not differ between patients with minor and major RAU either during acute phase or remission period, when compared to the control group. We found significantly higher salivary IgA level in patients with minor acute recurrent oral ulceration when compared to controls and also in comparison to the values of IgA in major acute RAU. Salivary IgA levels during the remission period both in patients with minor and major RAU, were normal. Natah[21] found that elevated levels of serum IgA and IgG were present in patients with recurrent aphthous ulcerations, and that salivary IgA did not differ in patients with RAU compared to the control group. Güven[22] also found elevated levels of serum IgA and IgM in patients with RAU. However the author did not find any changes in serum IgG levels. Porter et al.[13] described IgG subclass levels in the serum of patients with minor oral aphthous stomatitis, and concluded that there is no evidence to suggest that changes in IgG subclass are present in patients with RAU. In that study patients did not have active lesions of the oral mucosa. A more recent study by Vincente et al.[14] suggested that low serum levels of IgG2 in patients with acute ulcers might play an important role in the pathogenesis of RAU. The same authors also reported that serum IgG subclass level as well as total IgA may undergo changes which are dependant on different periods of activity and quiescence of the disease. Ben-Aryeh et al.[23] found that salivary IgA and serum IgA and IgG in patients with either dormant or acute RAU were within the physiologic range for healthy people. Authors[24] found lowered serum IgA in patients with acute RAU. Bennet and Reade[25] found that salivary IgA in patients with minor aphthous ulceration undependable of stage disease showed no deviation from the control group. Both in patients with minor and major RAU, during acute phase, remission and in controls salivary flow rate was within normal ranges and we can conclude that the quantity of saliva is unchanged in patients with RAU.

We can conclude that the local immunological defense response of saliva is activated in patients with minor RAU. Different values of serum immunoglobulin levels described by various authors could be due to the different disease stages of RAU investigated, as well as to the different technical methods employed. Further studies are needed in order to explain the etiopathologic mechanism in the development of RAU.

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S. Brozović

Department of Oral Medicine, School of Dental Medicine, Gundulićeva 5, 10000 Zagreb, Croatia

SERUMSKI IMUNOGLOBULINI A, G, M, TE SALIVARNI IMUNOGLOBULIN A U REKURENTINM AFTOZNIM ULCERACIJAMA

S A Ž E T A K

Za određivanje imunoglobulina A u slini korištena je tehnika radijalne imunodifuzije, a za određivanje serumskog IgA, IgG i IgM korišten je enzimski imunotest. Ispitivanje je izvršeno kod 30 bolesnika s rekurentnim aftoznim ulceracijama (RAU) u akutnoj fazi i tijekom perioda remisije te uspoređeno s nalazom 30 zdravih ispitanika. Značajno povišena razina IgA u slini ($p < 0.05$) pronađena je u bolesnika s minornim RAU u usporedbi s kontrolnom skupinom. Razina serumskog IgA povišena je u bolesnika s akutnim minornim RAU u usporedbi s kontrolnom skupinom. Razine serumskog imunoglobulina IgG i IgM nisu pokazale razlike između bolesnika s rekurentnim aftoznim ulceracijama bilo minor ili major i kontrolne skupine.