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Salivary Immunoglobulin A in Patients with Recurrent Aphthous Ulceration

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Abstract: It was hypothesized that serum level of immunoglobulins may play a role in the pathogenesis of oral mucosal diseases but little is known about the role of salivary immunoglobulins in the pathogenesis of these diseases. The aim of this study was to investigate the possible alterations in salivary immunoglobulin A (IgA-s) level in patients with oral aphthous ulcers and its relation with clinical parameters. Level of IgA was measured by "ELISA" test in the resting whole saliva of 24 patients with acute Recurrent Aphthous Ulceration (RAU) and during remission and the results were compared with 24 healthy volunteers. IgA was increased in acute RAU in comparison with healthy controls. Results demonstrated a significant increase in salivary IgA level in active lesions in relation to quiescence phase. No differences were found in salivary IgA level between major and minor acute RAU and other clinical parameters. The results of our study suggest a possible role of mucosal immune system in the pathogenesis of these lesions. It is reasonable to postulate that modulation of salivary immune system *in vivo* or production of immune materials *in vitro* can be efficient in the prevention or control of aphthous lesions.

Key words: Salivary immunoglobulin A, saliva, oral aphtha, aphthous ulcer, serum immunoglobulin A

INTRODUCTION

Recurrent Aphthous Stomatitis (RAS) is the most common cause of recurrent oral ulceration and affects approximately 20% of the general population (Greenberg and Glic, 2008). Its onset is usually during childhood, with a tendency to diminish in frequency and severity with age (Rogers, 1997). It has three clinical presentations: major, minor and herpetiform.

Major aphthae (sometimes referred to as periadenitis mucosa necrotica recurrence or Sutton's disease) accounts approximately 10 to 15% of RAS cases. They usually appear after puberty and are round or ovoid shape with clearly defined margins. They are usually deeper and larger and last longer than minor aphthae. They have a raised irregular border and frequently exceed 1 centimeter in diameter. They can last for weeks or months and often leave a scar after healing (Rogers, 1997).

Minor aphthae (also called Mikulicz's aphthae or mild aphthous ulcers) account for 75 to 85% of all cases of RAS. These ulcers are smaller than 8 to 10 mm and tend to heal within 10 to 14 days without scarring (Rogers, 1997).

Five to ten percent of all RAS cases are herpetiform ulcers (Rogers, 1997). Multiple (5 to 100) 1 to 3 mm

crops of small, rounded, painful ulcers resembling ulcers of herpes simplex are seen in the mucosa and lasts 10 to 14 days (Rogers, 1997).

Localized burning sensation or pain can proceed for 24 to 48 h before presenting ulcers. The lesions are painful, clearly defined, shallow, round or oval, with a shallow necrotic center covered with a yellow-grayish pseudomembrane and surrounded by raised margins and erythematous haloes. The pain lasts for three to four days, at which point early epithelialization can occur (Scully *et al.*, 2003).

The precise etiology of Recurrent Aphthous Ulcerations (RAU) remains unknown, although genetic susceptibility, infections and alteration in immune system has been considered as etiologic factors (Martinez *et al.*, 2007). Most of the oral inflammatory diseases seem to have a local origin, but systemic predisposing factors may also have a role in their pathogenesis (Sistig *et al.*, 2002). Local immune factors play a role in protection against oral diseases, which may be related to immunoglobulin A (IgA) responses (Kilian *et al.*, 1989).

IgA is the major class of immunoglobulin found in mucous secretions (Kilian *et al.*, 1996). Antibodies existing in mucosal secretions, especially IgA, are attached to microorganisms and reduce their mobility and

adhesive properties (Russmann *et al.*, 1999) and are considered as the first line of defense in oral cavity (McNabb and Tomasi, 1981).

Salivary immunoglobulin A is an excellent indicator of the oral mucosa immune status (Smith *et al.*, 1998; Hucklebridge *et al.*, 1998; Externest *et al.*, 2000). If we accept that the salivary immune system is actively involved in the pathogenesis of recurrent ulcers, measuring IgA-s changes may have a valuable role in prediction of the onset and management of the disease (Martinez *et al.*, 2007).

On the other hand, many authors reported no differences in total salivary IgA in either acute or dormant RAU stages with healthy persons.

Further, researches are needed to clarify the exact role of salivary immunoglobulins in immunopathogenesis and the clinical course of oral mucosal inflammatory disease.

MATERIALS AND METHODS

Informed consent, according to Helsinki II, was obtained from each participant prior to investigation. The study population consisted of 24 patients with minor (19 ulcers) and/or major (12 ulcers) ulcers attending in Oral Medicine Department of Mashhad Dental Faculty between July 2008 and May 2009. Most of our patients were male (14 cases). The age range was between 20-50 years with a mean age of 29.8 years (Table 1).

The saliva samples were collected in acute phase (within three days of ulcer onset) and in remission phase, (with clinically healthy oral mucosa). The aphthae were clinically classified. None of the patients with RAU received any systemic or topical medication 1 week prior to analysis (Martinez *et al.*, 2007). All patients were healthy and nonsmokers.

Control group included 24 healthy volunteers which were statistically matched with case group (13 men and 11 women, age range between 20-50 years, mean 28.46 years) and all of them were non-smokers and healthy.

The following data was collected by check list: the past medical history, type of aphthae (Minor, Major, herpiti-form), number of aphthous ulcerations, size of aphthous ulceration (measured by gauge), pain and burning rate (VAS), age and gender of patients, predisposing factors (stress, allergy to specific food

stuffs, gastrointestinal disorders, menstrual cycle and familial history), frequency of monthly recurrence, time (day) elapsed from appearance of ulceration and menstruation cycle for female patients.

Saliva collection: The method of Wu-Wang was used for saliva collection (Wu-Wang *et al.*, 1995). To avoid circadian variations, saliva samples were collected between 9 a.m. and 1.00 p.m. In order to obtain a sample of total saliva, the patients were instructed not to eat or drink (except water) for 1 h (Martinez *et al.*, 2007). Mouth washing with pure water was carried out right before sampling (Martinez *et al.*, 2007). All participants were instructed to collect saliva in their mouths for 5 min without swallowing and to spit into a clean plastic container. Saliva samples were kept in ice during the collection. In order to reduce bubble and foam, samples were centrifuged (Externest *et al.*, 2000) and stored at -70°C freezer until biochemical analysis.

Finally, levels of salivary immunoglobulin A were determined by enzyme-linked immunosorbent assay (ELISA) (Brody and Silberman, 1969) (salivary IgA Immuno diagnostic ELISA kit, Beta, Germany).

Statistics: We used one- way ANOVA, paired t-test, independent t-test and Pearson correlation coefficient or nonparametric equivalents for statistical analysis.

All results were considered significant for a significance likelihood below 5% (p<0.05) thus, yielding at least 95% of confidence in the conclusions presented.

RESULTS

The most prevalent clinical form of aphthae was minor ulcers (83.3%) and prevailed on the labial and buccal mucosa, with an average number of two lesions per patient.

Reported predisposing factors were: stress, allergy to food stuffs (29.2%), gastrointestinal disorders, menstrual cycle and familial history but in some cases no predisposing factors were identified.

Two female patients reported concurrency of menstruation cycle with aphthous ulcerations. Thirteen cases (54.2%) had a positive family history. Twenty nine percent of aphthous patients reported a history of allergy to specific food stuffs (such as pepper and melon) (Table 2).

Table 1: Distribution of patients at case and control groups based on sex

Sex	Case		Control	
	No.	(%)	No.	(%)
Male	14	58.3	13	54.2
Female	10	41.7	11	45.8
Total	24	100.0	24	100.0

Table 2: Distribution of case group based on history of allergy

History of allergy	Case group	
	No.	(%)
Positive	7	29.2
Negative	17	70.8
Total	24	100.0

Table 3: Comparison of mean IgA-s in case group in acute and remission phases

Case group	Mean	SD
Acute phase	170.4	109.5
Remission phase	111.2	96.1
p-value = 0.001		
T = 4.495		

Table 4: Comparison of mean IgA-s in case (acute phase) and control groups

Group	Mean	SD
Case (acute phase)	161.7	95.8
Control	121.9	109.9
p-value = 0.032		
Z = -2.145		

Table 5: Comparison of mean IgA-s in case (remission phase) and control groups

Group	Mean	SD
Case (remission phase)	111.2	96.1
Control	121.9	109.9
p-value = 0.956		
Z = -0.076		

The recurrence rate was from several times per month (in 7 patients) to once every month (in 8 cases) or once during several mounts (in 9 patients).

The mean level of salivary IgA in case group in acute phase (the presence of aphthous ulcers) and in remission phase was 161.7 and 111.2 mg mL⁻¹, respectively, but in control group the mean level was 121.9 mg mL⁻¹ (Table 3, 4).

The level of salivary IgA in case group in acute phase was significantly higher than control group (p-value = 0.02<0.05) (Table 4) but there was no significant difference between levels of IgA in aphthous patients at the second stage and healthy persons (p-value = 0.001>0.05) (Table 5).

Generally, there was no significant relationship between the age (p-value = 0.46>0.05) and sex (p-value = 0.24>0.05) of the patients with the levels of IgA, but correlation coefficient r = -0.108 revealed that levels of IgA reduce as age increases.

Based on the spearman correlation coefficient results (equaled to 0.089) there was no significant relationship between the number of ulcers and levels of IgA (p-value = 0.68>0.05).

The mean level of IgA in females with aphthous ulcerations at menstruation period (29 mg mL⁻¹) and without menstruation cycle (169.75 mg mL⁻¹) didn't show any significant difference (p-value = 0.11>0.05).

No significant difference was found in the level of IgA between different frequency of recurrences (several times per month = 189.1, once per month = 167.5, once per several months = 135.3).

Statistical analysis indicated no significant relationship between pain or burning sensation of aphthous ulcer and the levels of IgA (p-value = 0.72>0.05).

Pearson correlation coefficient was 0.076 that represented poorly positive relationship between severity of pain and levels of IgA (p-value = 0.72>0.05).

DISCUSSION

The etiology of Recurrent Aphthous Stomatitis (RAS) is not clear. A number of factors, such as emotional stress, local trauma and hormonal imbalances trigger the aphthae (Ship *et al.*, 1967; Amerongen and Veerman, 2002).

Immunoglobulin A (IgA) represents one of the five classes of human antibody (IgG, IgA, IgM, IgE and IgD). IgA antibody is produced more than any other subtypes during the day; it is the second most prevalent antibody in the serum and predominant in mucosal secretions.

It is generally accepted that saliva has an important role in maintenance of oral health. Numerous studies have shown that subjective and objective functional morbidity occurs in persons with insufficient saliva production. (Kakizawa *et al.*, 1973).

Salivary antibodies which are present in mucosal secretions, IgA in particular, combine with microorganisms to reduce their motility and adhesive properties (Russmann *et al.*, 1999). Many studies have demonstrated strong correlations between titers of specific IgA-s antibodies in secretions and resistance to infection (Russmann *et al.*, 1999). It seems that IgA-s has an immunoregulatory role and provides protection (Martinez *et al.*, 2007).

The aim of this study was to evaluate the concentration of IgA-s in aphthous patients and compare its level with healthy persons. Present results were compatible with the study of Kakizawa *et al.* (1973) and Brozovic *et al.* (2001), regarding the difference of salivary IgA levels between aphthous and non aphthous patients. Application of ELISA technique for determination of salivary IgA level is the preference of the present study in comparison to other researches.

In the study of Kakizawa, salivary IgA level was affected by menstruation cycle (Kakizawa *et al.*, 1973) but our study didn't confirm such a relationship.

In 2002, Sistig *et al.* (2002), assessed salivary IgA and IgA in oral mucosal diseases. Their results suggested that IgG and IgA levels increased in acute Recurrent Aphthous Ulcerations (RAU). In patients affected with RAU in remission period, IgG, IgG4 and IgA1 returned to normal values, while IgG2, IgG3 and IgA2 remained high.

Martinez *et al.* (2007), assessed secretory IgA level, total proteins and salivary flow in 20 aphthous patients. The result of their study was similar to our study.

In the study of Ben-Aryeh *et al.* (1976) a significant increase in salivary IgA level in the presence of aphthous ulcer was observed but there was no significant difference in salivary IgA in the absence of aphthous ulcer in case and control groups.

However, some studies found no difference in amount of this salivary immunoglobulin between patients with and without aphthous ulcers such as the study of Bennet and Reade (1982). They evaluated salivary immunoglobulin A level in normal subjects, tobacco smokers and patients with minor aphthous ulceration and no significant difference observed (Bennet and Reade, 1982).

On the other hand, the study of Sistig *et al.* (2002) showed normal values of IgG1, IgG4 and IgA1 in patients with RAU in remission period, while IgG2, IgG3 and IgA2 level was increased. The authors believed that this could be related to the chronic antigenic stimulation, because of the high concentration of the specific microorganisms which could be involved in the pathogenesis of RAU and leads to increased specific immunoglobulin. Sistig *et al.* (2002) believed that changes in specific IgA subclasses in aphthous patients could not be demonstrated by simple determination of total IgA.

They found no difference in salivary IgA 1-2 and IgG1-4 levels between minor and major aphthae that is in contradiction with the results of our study. They concluded that increased IgG levels in acute RAU might support an infective causative agent in the development of RAU. They also believed that salivary IgA and G subclasses are implicated in some oral inflammatory diseases and may play an important role in their defense mechanisms and/or reflect clinical changes in these conditions results of this study suggests a more important role for immunoglobulin G.

As we couldn't find any significant relation between major and minor aphthous ulcers with the level of salivary immunoglobulin A, it seems that this factor is not important in clinical presentation of RAU.

The results of this study suggest that the salivary IgA is implicated in RAU and may play an important role in their defense mechanisms. In patients with RAU, IgA was increased when compared with the healthy controls. This could implicate an important role of salivary immunoglobulins in pathogenesis of RAU, although our results are difficult to compare because of the lack of sufficient data on salivary immunoglobulin subclasses in RAU.

CONCLUSIONS

Although, increased salivary immunoglobulin A level has been found in active aphthous ulcerations, the exact protective role of this immunoglobulin is still not clearly defined.

If we accept the immunoregulatory role of IgA-s in the pathogenesis of aphthae, it is logical to provoke the immune system to prevent the recurrence of these lesions.

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