Concentration of α1-antitrypsin in serum of blood in dependence on the level of intensity of denture stomatitis

ELŻBIETA MIERZWIŃSKA-NASTALSKA, MARTA JAWORSKA, URSZULA DEMKOW

[1]Department of Prosthetic Dentistry, Medical University of Warsaw, Poland, [2]Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Poland

Abstract

Many authors demonstrated beneficial role of some acute phase proteins in response to tissue damage due to inflammatory reaction, injury or infection. Relationship between acute phase protein concentration and intensity of pathologic process has been demonstrated.

The aim of the study was to evaluate serum α_1-antitrypsin (AAT) concentration in patients with denture stomatitis as well as in healthy subjects. AAT concentration was measured by radial immunodiffusion method using NOR Partigen plates manufactured by Behring (USA). Study results showed highly significantly increased concentration of plasma AAT in denture stomatitis patients in comparison to the controls. Plasma α_1-antitrypsin concentration in patients with severe, diffuse inflammation in oral mucosa over denture foundation area was highly significantly higher than in moderate, local inflammation group and than in the group of patients with hyperplastic lesions.

Key words: *denture stomatitis, α_1-antitrypsin, Newton classification*

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Introduction

Denture stomatitis is frequently diagnosed condition in patients using removable acrylic dental prostheses. Clinical signs include various degree of stomatitis. In mild form of stomatitis local pin-pointed lesions are present. In generalized form lesions over the surface of mucosa adhesing to denture plate are observed. Papillary type lesions are most frequently localized in the medial part of hard palate, over denture relief area. Local lesions may by accompanied by symptoms: oral cavity soreness, burning and stinging sensations. Sometimes angular stomatitis and white biofilm covering mucosal surface is also present.

Important causes of denture stomatitis include dentures trauma, Candida infection and hygienic negligence. Considerable risk factor is 24-hour wearing of dentures without night-time interval and keeping the dentures in liquid environment out of the oral cavity. Poor hygienic habits concerning acrylic dentures and oral cavity enhance dental plaque formation, remaining of food debris and bacterial growth under denture plate. Nutritional deficiencies also affect formation and course of denture stomatitis. Iron and B-group vitamins deficiency, high-carbohydrate diet enhance disease development [1, 2].

Important medical reasons for denture stomatitis include systemic and metabolic diseases, immunodeficiency states, general exhaustion and some therapeutic agents like antibiotics, corticosteroids, immunosuppressants, radiotherapy and chemotherapy [3, 4].

Response of the organism to tissue damage caused by inflammation, injury or infection results in rapid increase in protein production. These are acute phase proteins, which include α_1-antitrypsin (AAT). Alpha1-antitrypsin is elastase inhibitor protecting from tissue damage mediated by phagocytes hydrolase secreted during inflammatory reaction [5-7].

Many authors report that acute phase proteins secretion, concentration and activity depend on the form and activity

Correspondence: Marta Jaworska-Zaremba, Katedra Protetyki Stomatologicznej AM IS, ul. Nowogrodzka 59, 02-006 Warszawa, tel. +48 22 502 18 86, e-mail: barmeza@o2.pl

of disease. The main stimulator and regulator of acute phase proteins synthesis in human is interleukin-6 (IL-6) [8-10].

Aim

The aim of the study was the evaluation of α_1-antitripsin serum concentration in patients with denture stomatitis at various stage of the disease as well as in healthy subjects.

Material and methods

Study group included 80 patients (51 women, 29 men) aged 48-86 years (mean 67 years) wearing partial and complete dentures.

The study was based on clinical assessment of the oral mucosa, lips and lip corners. Intensity of pathologic changes was evaluated according to modified Newton classification. Stage I include appearance of a few pin-pointed erythematous lesions, in stage II diffuse inflammation confined to the mucosa in contact with the denture base is present, stage III includes hyperplastic lesions. Stage 0 includes patients with no pathological lesions in oral mucosa. The presence of sores, white mucosal biofilm and angular stomatitis was also taken into consideration. Condition of removable dentures was checked, its appearance, mucosal surface coarseness, occlusal vertical dimension and fixing. Denture hygiene was evaluated using modified plaque index according to Ambjőrsen.

Patients were interviewed about their dentures wearing habits (24-hours, overnight interval), how they keep their prostheses outside the oral cavity (in dry environment, in water). Patient's general condition was also taken into consideration including medical history, presence of chronic diseases, medications and oral symptoms: soreness, burning sensation, dryness, taste disorders.

Mycological tests were performed by culturing direct oral mucosa and tissue-fitting surface of dentures swabs samples on Sabouraud agar followed by 48-72 hours incubation period in temperature 37°C and assessment of yeast-like colony growth.

Based on patients' interview, clinical evaluation and mycological tests results, denture stomatitis in the oral mucosa was diagnosed in 56 patients. Then a group of 30 patients in various disease stage and clinical appearance of denture stomatitis was selected for further evaluation. None of them had concomitant medical condition nor was on chronic medication.

Control group included 10 subjects (5 women, 5 men) in good general health condition, wearing removable dentures with no pathologic changes in the oral mucosa and negative fungal cultures. Informed consent for blood drawing was obtained from each subject according to an institutional review board-approved protocol.

All the patients were drawn a 10 ml of venous blood, blood sample was centrifuged at 2500 RPM for 15 minutes. Serum α_1-antitripsin concentration was measured by using radial immunodiffusion method. Collected serum was portioned by gel diffusion method using NOR Partigen plates manufactured by Behring (USA). Plate wholes were filled with 0,005 ml samples of examined serum and equal volume of control plasma (Behring). Plates were kept in humid chamber in ambient temperature. Results were read after 24 hours. Diameter of the precipitation was assessed by using ready-made matrix, then α_1-antitripsin concentration in IU/ml was read from the datasheet. Statistical analysis of results was performed using t-Student test.

Results

The most frequent systemic factors favouring denture stomatitis were chronic diseases, especially diabetes and antibiotic treatment. The most frequent local factors were poor denture hygiene, continuous using of dentures, improper keeping outside oral cavity, improper denture fixation (tab. 1). Clinical evaluation of patients with denture stomatitis revealed

Table 1. Systemic and local factors favouring development of denture stomatitis

Systemic factors	Percentage of patients	Local factors	Percentage of patients
diabetes	8.5%	24-hours wearing of dentures	57.4%
chronic diseases	12.7%	improper keeping of dentures outside oral cavity	75.0%
immunodeficiency states	2.1%	poor denture hygiene	87.2%
nutritional disorders	3.2%	improper denture stabilization	42.6%
neoplasms	5.3%	denture rebasing	14.3%
radiotherapy	2.1%	denture coarseness	21.8%
antibiotic treatment	7.4%	improper occlusal vertical dimension	28.9%
chemotherapy	2.1%		
corticosteroids	1.1%		
other	3.2%		

various intensity of changes. Serious, diffuse inflammation (stage II) was diagnosed in 25 subjects (31.25%), moderate inflammation (stage I) in 17 subjects (21.35%). Hypertrophic lesions (stage III) was present in 14 patients (17.5%). White biofilm layer and sores were diagnosed in 15% and angular stomatitis in 20%. In same cases (35%) local changes were accompanied by oral symptoms (soreness, burning, dryness, taste disorder) (tab. 2).

Mycological tests revealed presence of yeast-like fungi in oral mucosa swab in 56 patients (70%) and presence of yeast-like fungi in denture surface swab in 72 patients (90%) (tab. 3).

The mean serum AAT concentrations in denture stomatitis patients in relation to oral mucosa lesion stage are shown in table 4. AAT concentration in patients with serious, chronic, diffuse inflammatory changes in denture foundation area mucosa (stage II) was highly significantly higher in comparison to mild degree disease patients (stage I) and to papillary hyperplasia patients (stage III). All groups of patients presented AAT levels highly significantly higher than the controls.

Discussion

Recent studies demonstrated that denture stomatitis develop as a result of simultaneous action of several factors. Interaction between denture plaque microorganisms, broken continuity of oral mucosa due to injury and immune response of the host leads to inflammatory reaction in oral mucosa. The common element of that three damaging processes is rapid increase in production of proteins called acute phase proteins.

Table 2. Clinical study results

Clinical evaluation		**Number of patients (%)**
oral mucosa changes (inflammation degree)	stage 0	24 (30%)
	stage I	17 (21.25%)
	stage II	25 (31.25%)
	stage III	14 (17.50%)
angular chealitis		20%
white biofilm, sores		15%
soreness, burning, dryness, taste disorder		35%

Table 3. Mycological test results depending on place of swab taking

		Mycological test results			
		mucosa swab		**denture swab**	
women	men	+	-	+	-
51	29	56 (70%)	24 (30%)	72 (90%)	8 (10%)
total: 80					

Table 4. Mean serum α_1-antitrypsin concentration in denture stomatitis patients

Mucosal inflammation stage	**Number of patients**	**α_1-antitripsin concentration IU/ml ± SE**
moderate inflammation stage I	9	178.7±10.9***
diffuse inflammation stage II	12	250.6±8.1***
hyperplastic lesions stage III	9	169.1±8***
no oral mucosa inflammation control group	10	110.9±4.2

**** p<0.001*

Acute phase reaction initially local, then systemic process, is part of non-specific immunity of the organism. This is the earliest response to tissue damage and include rapid increase in production of numerous proteins, called acute phase proteins [6, 7]. The main stimulant for their synthesis (mostly hepatic) is interleukin 6 [11]. Alpha$_1$-antitrypsin is one of the acute phase protein. AAT production is not affected by IL-1 and TNF-α. AAT belongs to protease inhibitors, enzymes that prevent tissue damage related to proteolytic enzymes action secreted during inflammatory reaction [12]. AAT has a broad anti-inflammatory spectrum. Although AAT is mainly produced in the liver, its main function is to protect the lung against proteolytic damage from neutrophil elastase. Individuals with AAT deficiency may develop emphysema, cirrhosis, panniculitis, and Wegener's granulomatosis. AAT may also exhibit anti-inflammatory activity unrelated to inhibition of serine proteases. *In vitro*, AAT inhibited release of inflammatory cytokines (TNF alpha, IL-1 beta) from human monocytes stimulated with LPS, but enhanced the release of anti-inflammatory cytokine, IL-10 [13-16].

In vitro studies were performed to determine changes in cytokines (IL-1, IL-6, TNF-α) and acute phase proteins concentrations following cancer of gastrointestinal tract and liver resection. Protein level: α_1-antitrypsin, C-reactive protein and haptoglobin significantly increased postoperatively [10]. Meki at all [8], in children intoxicated with scorpion venom there were found increase in cytokines concentration, mainly IL-6 and AAT in proportion to intoxication severity. Implementation of sensitive enough diagnostic methods allowed for measurement of active cytokines (TNF-α, IL-6, IL-8) and AAT concentration in complicated pancreatitis cases [17].

Results of our study revealed highly significantly increased serum AAT concentration in all groups of patients in comparison to the controls. The highest values were observed in patients with severe diffuse inflammation in oral mucosa of denture foundation area. Above results suggest existing of correlation between acute phase protein concentration and intensity of oral mucosa inflammation. High AAT concentration was found in patients with intensive growth of fungi confirmed by mycological tests.

Conclusion

We have found that AAT concentration parallel clinical course of denture stomatitis. Diagnostic value of AAT concentration measurement may have prognostic value for this disease staging.

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