® Postepy Hig Med Dosw (online), 2011; 65: 40-45
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2010.12.09
Accepted: 2011.01.24
Published: 2011.02.01

Impact of salivary flow and lysozyme content and output on the oral health of rheumatoid arthritis patients

Wpływ przepływu, stężenia i „wyrzutu" lizozymu w ślinie na zdrowie jamy ustnej pacjentów reumatoidalnych

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Summary

Purpose: The aim of the study was to examine salivary flow rate, DMF index, lysozyme concentration and its output in two groups of rheumatoid patients and to compare the results with those of healthy controls.

Material/Methods: Rheumatoid arthritis (RA) patients were divided into two study groups: with reduced salivary flow rate ≤0.15 ml/min (RA HS, hyposalivation) and with normal salivary secretion rate >0.2 ml/min (RA NS, normal salivation). The healthy control group (C) was recruited from the Department of Conservative Dentistry. Salivary lysozyme concentration was determined by radial immunodiffusion. ANOVA followed by LSD test were used for the statistical analysis.

Results: We found that lysozyme concentration was higher and lysozyme output and salivary flow rate were statistically lower in the RA HS group in comparison to the RA NS and C groups. The DMF index was statistically higher in both RA groups in comparison to the control group.

Conclusions: RA disease impacts negatively on oral health and salivary parameters. Hyposalivation of RA patients increases the negative influence of RA on oral health. RA patients should receive more stomatological attention.

Key words: **saliva • lysozyme • salivary flow rate • DMF (decayed, missing, filled teeth) • rheumatoid arthritis**

Streszczenie

Cel: Tematem pracy była ocena i porównanie szybkości przepływu śliny, wskaźnika PUW (wskaźnik zębów próchnicowych, usuniętych z powodu próchnicy i wypełnionych), stężenia i wyrzutu lizozymu pomiędzy dwiema grupami pacjentów reumatoidalnych i w grupie kontrolnej.

Materiał/Metody: Pacjenci reumatoidalni podzieleni zostali na dwie grupy: ze zredukowanym przepływem śliny ≤0,15 ml/min (RZS z hiposaliwacją) i z prawidłowym przepływem śliny >0,2 ml/min (RZS bez hiposaliwacji). Grupę kontrolną stanowili pacjenci Zakładu Stomatologii Zachowawczej. Stężenie lizozymu oznaczono metodą immunodyfuzji radialnej. Analizę statystyczną przeprowadzono testem ANOVA i testem NIR.

Rezultaty: Wykazano, że w grupie pacjentów z RZS ze zmniejszonym przepływem śliny stężenie lizozymu było wyższe, zaś jego wyrzut i przepływ śliny niższe w porównaniu do grupy bez zaburzeń saliwacji i w grupie kontrolnej. Wskaźnik PUW był statystycznie wyższy w obu grupach badanych w porównaniu do grupy referencyjnej.

Wnioski: Choroba reumatoidalna negatywnie wpływa na zdrowie jamy ustnej i wskaźniki śliny. Hiposaliwacja występująca u pacjentów reumatoidalnych potęguje negatywny wpływ choroby reumatoidalnej na zdrowie jamy ustnej. Pacjenci z RZS powinni zostać objęci specjalną opieką stomatologiczną.

Słowa kluczowe: **ślina • lizozym • przepływ śliny • PUW • reumatoidalne zapalenie stawów**

Full-text PDF: http://www.phmd.pl/fulltxt.php?ICID=932232

Word count: 2554
Tables: 5
Figures: –
References: 21

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory musculoskeletal disease with considerable morbidity and mortality [15] and may present with extra-articular manifestations including involvement of exocrine lacrimal and salivary glands [2]. The decrease of salivary function in RA is assumed to be related to the lymphocytic infiltrate present in affected glands and manifested in decrease of salivation and chemical changes [2].

Hyposalivation (HS) is a condition associated with both a decrease in the amount of saliva produced and an alteration in its chemical composition. HS has a deleterious effect on many aspects of oral function and general well being. It can cause a significant decline in quality of life by decreasing taste sensation and impairing chewing ability [5]. Furthermore, it may alter regular eating patterns, reducing the pleasure of eating due to impaired or diminished taste sensation. Patients with hyposalivation often report avoidance of some foods, such as dry and sticky foods, due to the inability to chew or swallow effectively [5]. Also, hyposalivation may impair a patient's ability to speak, and cause cracks and fissures in the oral mucosa as well as halitosis [5]. It can cause denture wearing to be very uncomfortable, exacerbating chewing difficulties [5]. This is mainly due to reduced surface tension between the dry mucosa and the denture. Hyposalivation can affect numerous aspects of oral function, contributing to pain, caries and oral infections [5].

Saliva serves a number of functions. Firstly, it acts as the first defense against mechanical, chemical and infectious attacks by protecting against numerous oral bacteria and fungi [6]. By providing local antimicrobial activity through enzymes such as immunoglobulins [9], lysozyme, lactoperoxidase [9] and histatins [21], saliva aids in reducing oral infections. It acts as a vehicle for nutrients and digestive enzymes and assists in the preparation of the food bolus [10]. It also functions to maintain tooth integrity from dental decay by supporting ongoing remineralization of teeth [10]. It does so by providing a reservoir of calcium and phosphate ions and forming the glycoprotein pellicle. It also physically protects teeth from harmful substances by coating them with glycoproteins and mucoids. This also assists in oral lubrication [10]. In addition, saliva functions in maintaining a neutral oral pH through bicarbonate and phosphate buffer systems. Chewing, swallowing and speech are all facilitated by saliva [10].

Proteins – lysozyme, lactoferrin, and lactoperoxidase – working in cooperation with other components of saliva can have an immediate effect on oral bacteria, interfering with their ability to multiply or killing them directly. Some studies have shown that one source of lysozyme is neutrophils, others that monocytes and macrophages are the dominant contributors to the lysozyme content in serum [8,20]. Lysozyme is an enzyme that hydrolyses glycosidic bonds and is thus able to hydrolyze the cell wall peptidoglycans and some microorganisms and thereby kill the organism [19]. Lysozyme can cause lysis of bacterial cells, especially *Streptococcus mutans*, by interacting with anions of low charge density, chaotropic ions and with bicarbonate. The cooperation of the above ions leads to destabilization of the cell membrane, probably through the activation of autolysins [16]. Lysozyme also appears to alter intermediary glucose metabolism in sensitive bacteria and, in some cases, to cause aggregation perhaps contributing to clearance of bacteria from the oral cavity. Its ability to bind to hydroxyapatite suggests an antimicrobial role on the tooth surface [6].

Although salivary gland involvement in RA has been known for a long time, it did not draw much attention, from either clinicians or researchers.

The aim of the study was to examine lysozyme concentration and lysozyme output, as well as salivary flow rate, which can directly reflect salivary gland function, and DMF index (which reflects oral health), in RA NS and RA HS groups of patients.

Patients and Methods

The study was approved by the Ethics Committee of the Medical University of Bialystok. All persons gave informed, written consent. RA patients were selected from the Department of Rheumatology, Medical University of Bialystok. All RA patients fulfilled the revised criteria according to the American College of Rheumatology [3]. Our RA group included subjects between 32 and 76 years of age, disease duration 0.5–25 years. They had to have at least five teeth. All subjects were periodontally healthy, pocket depth <3 mm; GI from 0 to 1.875. Patients were also excluded if they used medications which could cause oral or ocular dryness. None of the RA patients had diabetes mellitus or hypertension; none of the subjects were smokers.

RA patients were divided into two study groups: I (24 subjects) with reduced salivary flow rate ≤0.15 ml/min, termed the hyposalivation group (RA HS), mean age 50.26 years; and II (32 subjects) with normal salivary secretion rate >0.2 ml/min, termed RA NS, mean age 51.47 years [17]. An age-, sex- and stomatologically matched healthy control group (C) (30 volunteers, mean age 53.94 years) was recruited from the Department of Conservative Dentistry.

A check-up of the oral cavity was done in artificial light by using diagnostic dental tools (dental mirror and probe). Following WHO criteria, the caries status in all individuals was determined by the same dentist using the DMF index.

Persons were instructed to refrain from food and beverages, except water, for two hours before saliva collection. All salivary samples were collected between 8 a.m. and 10 a.m., so that circadian influences would be minimized. The secretion rate of resting whole saliva (RWS) was measured for 15 min. The volume of each sample was measured by a pipette with reading accuracy of 0.01 ml and the flow rate was determined from volume divided by the time (in minutes) for sample collection. Salivary flow rate was expressed as the volume of saliva secreted per minute. After measuring volumes salivary samples were centrifuged at 3,000 × g for 20 minutes at 4°C to remove cells and debris. The resulting supernatants were divided, frozen and kept at –80°C until analyzed.

Determination of salivary concentration and output of lysozyme

Salivary lysozyme concentration was determined by radial immunodiffusion (The Binding Site, UK) as described initially by Mancini, Carbonara and Heremans [11]. 10 µl of appropriate diluted saliva, undiluted control serum and calibrators were placed into agarose gel immunodiffusion plates containing the monospecific antibody. The plates were then tightly closed, re-sealed in their original pouches and placed into the moist chamber. After 75 hours of incubation at room temperature the precipitation ring diameters were measured using the Digital RID Plate Reader and analyzed using *RID*Read software. Lysozyme output was expressed in mg/ml and lysozyme concentration in mg/ml.

The analyses of each saliva sample were performed in duplicate.

Table 1. Salivary flow rate in RA patients

	RA NS N=30	RA HS N=30	Control N=32
RA NS M=0.4885		0.000000	NS
RA HS M=0.089	0.000000		0.000000
Control M=0.4715	NS	0.000000	

Table 2. Lysozyme concentration (mg/ml) in RA patients

	RA NS N=30	RA HS N=30	Control N=32
RA NS M=0.0421		0.0000	NS
RA HS M=0.1766	0.0000		0.000000
Control M=0.0336	NS	0.0000	

Statistics

ANOVA followed by LSD test were used for the statistical analysis. To show correlation Pearson test was used. Results were expressed as the mean and SD. A level of $p \leq 0.05$ was considered to be significant. Statistical analysis was carried out using STATISTICA Version 6.

Results

Table 1 shows salivary flow rates in RA patients. The lowest salivary flow rate was observed in the RA HS group. It was 5.49 and 5.29 times lower in comparison to the RA NS and control groups (p=0.000000). Salivary flow rate in RA NS patients showed a tendency to increase, in comparison to controls.

The lysozyme concentration was the highest in the RA HS group: 4.25 times higher than in RA NS and 5.25 times higher than in the control group (p=0.0000 and p=0.000000 respectively). The lysozyme concentration in RA NS showed a tendency to increase in comparison to the control group (Table 2).

The lysozyme output in all groups ranged from 0.0153 to 0.0167 (mg/min) and was statistically lower in the RA HS group than in the RA NS and control groups (p=0.0311 and p=0.00185 respectively). The lysozyme output tended to decrease in RA NS in comparison to controls (Table 3).

The DMF index was statistically higher in the RA HS group than in the RA NS and control groups (p=0.037 and p=0.000001 respectively). The DMF index was 1.229 times higher in RA NS in comparison to controls (p=0.000259) (Table 4).

Table 3. Lysozyme output (mg/min) in RA patients

	RA NS N=30	RA HS N=30	Control N=32
RA NS M=0.0164		0.0311	NS
RA HS M=0.0153	0.0311		0.00185
Control M=0.0167	NS	0.00185	

Table 4. DMF index in RA patients

	RA NS N=30	RA HS N=30	Control N=32
RA NS M=22.811		0.037	0.000259
RA HS M=25.667	0.037		0.000001
Control M=18.559	0.000259	0.000001	

Table 5. Correlation between salivary flow, lysozyme concentration and its output in RA patients

		Salivary flow		Lysozyme concentration		Lysozyme output	
		r	p	r	p	r	p
RA NS	DMF	–0.04	0.824	0.05	0.785	0.04	0.828
RA HS		–0.09	0.667	0.1	0.65	0.15	0.491
Control		–0.11	0.531	0.18	0.307	0.1	0.574

Pearson test did not show any statistical correlation between salivary flow rate, lysozyme concentration and its output, and DMF index (Table 5).

Discussion

In the present study the main goal was to characterize salivary and oral differences between RA NS and HS patients and to compare the results with those of healthy controls.

When comparing both RA and control groups, a significant difference regarding salivary fluid secretion was seen only in the RA HS group. In the case of RA NS we noted an insignificantly higher salivary secretion rate in comparison to healthy controls. Similar results were obtained by Mignonga et al. [13]. These authors concluded that the lymphocytic infiltration, in the early phase of RA disease, might cause via cytokine secretion alteration of salivary gland function, which clinically might be expressed as increased salivary flow. Only destruction of the glandular acinar units might occur subsequently, leading to a reduction of salivary secretion. It is believed that B and T lymphocytes infiltrate the salivary glands, causing cell-mediated destruction of glandular elements; secretion of cytokines; production of autoantibodies that interfere with muscarinic receptors; and secretion of metalloproteinases that interfere with efficient glandular functions [12]. The release of acetylcholine from parasympathetic nerves initiates the stimulus-secretion coupling in the acinar cells of the salivary glands. The release of acetylcholine is brought about by an increase in the intracellular calcium level that ends in activation of the calcium-dependent K^+ and Cl^- channels. Disruptions of any of these steps will bring a decrease of fluid secretion [12]. The B and T lymphocytes which infiltrate salivary glands produce autoantibodies directed against the muscarinic receptor which are thought to play a role in inducing sicca symptoms.

As we mentioned, dental caries is the most common consequence of hyposalivation.

We found that RA HS had a higher DMF index than in the control group. However, the same results were obtained for RA NS, but the DMF index was significantly elevated in RA HS in comparison to RA NS. We did not reveal a significant correlation between salivary flow rate, lysozyme concentration and its output, and DMF index. However, a relationship between salivary flow rate and DMF was negative in RA patients, so the low flow rate can facilitate development of caries. Moreover, the saliva of patients with hyposalivation becomes more viscous and foamy, losing its lubricating ability and adhering to teeth and mucous membranes [5]. Also accumulation of plaque and an increase in the number of microorganisms in the saliva could be expected when the salivary secretion is low. With increasing plaque thickness, there is usually a simultaneous increase in the proportion of anaerobic Gram-negative bacteria. The sugar clearance time is prolonged and the buffering capacity decreased when secretion rates are too low, and so an increase in aciduric microorganisms could be expected. Also, access to antimicrobial substances is decreased when salivary secretion decreases. This might be followed by an increase in microbial species associated with opportunistic infections, as well as caries [1,14]. The high DMF index in RA NS might reflect infraclinical involvement of the salivary glands resulting in changes in saliva biochemistry long before development of hyposalivation.

Results obtained by Almstähl et al. [1] showed that dental status in subjects with hyposalivation was similar to that in the control group. Similar results were obtained by Beighton et al., who found no increase in the frequency of root caries in subjects with reduced saliva flow caused by medicines [4]. The study of Pedersen et al. [14] showed that patients

with pSS (primary Sjögrens' syndrome) had a significantly higher DMFS (surface DMF) compared to healthy controls, despite the fact that they brushed their teeth with toothpaste containing fluoride and visited their dentist more frequently than healthy controls. Pedersen [14] claimed that pSS patients harbor higher numbers of cariogenic and acidophilic microorganisms such as *Streptococcus mutans* and *Lactobacillus species* than healthy controls, and this could be a reason for the higher DMFS index.

Our results showed statistically higher concentration of salivary lysozyme in the RA HS group and a tendency to increase of this parameter in the RA NS group in comparison to the control group. The increase of lysozyme concentration is probably due to the decreased volume of salivary liquid. Infiltration of the salivary glands with lymphocytes could be another reason for the increased total salivary lysozyme level [7]. The latest hypothesis can be explained by the fact of increased secretion of lysozyme from leucocytes into the saliva. The study of Syrjänen et al. [18] showed an elevated lysozyme level in saliva of RA patients. They also reported that lysozyme immunoreactivity was observed in the serous acinar cells and in the epithelium of intercalated ducts, but never in the striated ducts. No difference in the localization or intensity of the staining could be found between RA and control series. The percentile distribution of B and T and monocytes cells was equal in both series. There were fewer monocytes, which are the main source of lysozyme, in glands showing lysozyme immunoreactivity-negative glands. This is evidence against the suggested direct relationship between monocyte cell counts and lysozyme concentration in external secretions.

From the clinical standpoint, total output rather than the volume of salivary lysozyme is important, as output reflects the total lysozyme secreted into the oral cavity. In our study, in the hyposalivation group we noted significantly lower output of lysozyme. The lower output can be explained by decreased synthesis or secretion of lysozyme. This fact may be a result of damage of salivary glands by infiltrates of lymphocytes followed by fibrosis, acinar atrophy and lymphoplasma cell adenitis. It is also interesting to note that output of lysozyme of RA NS showed a tendency to decrease in comparison to the control group. This seems to confirm the hypothesis that in the initial phases of RA, when lymphocytic infiltration has not resulted in significant loss of secretory cells, sialometry might not show any downregulation of salivary flow rate, whereas its composition may already have been changed by autoimmune inflammation. Our results suggest that RA patients who experienced either severe reduction in the salivary flow rate in general or reduction in the total amount of salivary lysozyme secreted into the oral cavity in particular are significantly less protected against damage to teeth and walls of the oral cavity than healthy persons.

Conclusions

It seems appropriate to claim that both qualitative and quantitative measurements of saliva should be performed in RA patients for a proper evaluation of the influence of RA on salivary function. RA disease impacts negatively on oral health and should receive increased stomatological attention, as the life of a patient already having various disabling disease manifestations is further complicated by stomatological problems.

It seems to be necessary to activate or enhance the host defense of saliva in RA patients. This can be attempted in a number of ways: by increasing the salivary flow rate and buffer effect; by adding lactoperoxidase system-, lactoferrin-, and lysozyme-containing products; or by immunization regimens. Moreover, these patients should receive an individual dental care program in terms of oral hygiene instructions, professional oral hygiene regimens, fluoride treatment, dietary supervision and frequent dental follow-up visits in order to prevent accelerated caries development.

Acknowledgements

All authors provide a statement concerning any commercial associations or patent licenses that might result in a conflict of interest with the work presented in the submitted paper.

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The authors have no potential conflicts of interest to declare.