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Sijalokemijski nalaz za bolesnike s oralnim lihen planusom

Sialochemistry in Patients with Oral Lichen Planus

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Sažetak

Svrha: Zna se da određeni sastojci sline mogu biti poremećeni kod bolesnika koji pate od oralnoga lichen planusa (OLP-a), no rezultati objavljenih istraživanja nisu konzistentni. Svrha ovog istraživanja bila je odrediti koncentraciju salivarnih analita u sklopu salivarnih enzima koji održavaju integritet oralnih sluznica, a on je oštećen kod oboljelih od oralnoga lichen planusa. **Ispitanici i postupci:** Skupini od 25 bolesnika s OLP-om (73±1,4 godine) i 24 kontrolna ispitanika (24±3,7 godina) određeni su u slini ukupni proteini, amilaza, magnezij, kalcij, bakar, klorid, fosfat i kalij. Ukupni proteini određeni su kolorimetrijskom metodom uz pirogalol. Natrij, kalij i klorid izmjereni su indirektnom potenciometrijom, bakar i magnezij atomskom apsorpcijskom spektrofotometrijom, a fosfat kolorimetrijskom uz pomoć molibdata. Statistička analiza obavljena je uz pomoć $\chi 2$ testa, Mann–Whitneyeva U-testa, analizom kovarijance i Spearmanovom korelacijom. **Rezultati:** Znatno povišene vrijednosti salivarnog klorida pronađene su kod oboljelih od OLP-a u odnosu prema sudionicima u kontrolnoj skupini (p=0,025). Nakon toga, kada su dobiveni rezultati prilagođeni s obzirom na količinu izlučene sline, ustanovljene su povišene vrijednosti salivarnog kalija, natrija, klorida i ukupnih proteina kod oboljelih od oralnoga lichen planusa u odnosu prema kontrolnoj skupini (p=0,622; p=0,504; p=0,00; p=0,586). Koncentracija salivarnih analita nije bila povezana s pušenjem. **Zaključak:** Povišene vrijednosti salivarnog natrija, kalija i klorida vjerojatno su posljedica velikih razlika u količini izlučene sline između oboljelih od OLP-a i sudionika u kontrolnoj skupini i ne upućuju na pojačanu antimikrobnu aktivnost kod bolesnika s OLP-om.

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Ključne riječi
slina; lihen planus; proteini i peptidi sline

Uvod

Oralni lihen planus (OLP) autoimuna je bolest posredovana T-limfocitima koja može zahvatiti i oralne sluznice. Ipak, etiopatologija bolesti nije jasna (1). No, dobro je poznata povezanost između normalnog sastava sline i njezine sposobnosti da obavi mnoge zadaće poput obrane od mikrobnih, gljivičnih i virusnih infekcija, zatim u zaštiti tvrdih i mekih tkiva, kemosenzornoj zaštiti te govorne i prehrambene funkcije sline (2). Poznato je da se sastojci sline mogu promijeniti zbog mnogih oralnih i sistemskih bolesti te lijekova, ali mogu biti i posljedica smanjenog izlučivanja sline i starenja. Gandara i suradnici (3) pokazali su da količina i sastav sline mogu biti promijenjeni kod oboljelih od OLP-a. Osim toga zna se da kada je smanjeno izlučivanje sline, tada je ona koncentriranija i njezina su reološka svojstva kompromitirana, što na neki način ograničava zaštitnu sposobnost sline, odnosno enzime, imunoglobuline i druge čimbenike (4).

Salivarni elektroliti utječu na mnoge enzimatske procese koji sudjeluju u obrani unutar usne šupljine, kao npr. hijaluronidaza, superoksidna dizmutaza, histatini i laktoferin. Smanjeni obrambeni kapacitet sline ima veliku kliničku važnost s obzirom na oralne bolesti (5).

Introduction

Oral lichen planus (OLP) is a T-cell mediated autoimmune disease which affects oral mucosa. However, its ethiopathology is poorly understood (1). The association between normal composition of saliva and its ability to fulfil various roles such as defence against microbial, fungal and viral infections, protection of oral hard and soft tissues and chemosensorial, speech and nutritional functions is well known (2). It is well known that salivary constituents might be disturbed due to various oral and systemic diseases as well as medications, but also as a result of decreased flow rate and aging. Gandara et al. (3) authors suggested that salivary flow rate and composition might be disturbed in OLP patients.

It is known when salivary secretion is reduced in volume, saliva is highly concentrated with its rheological properties being compromised. This limits the protective capacity of saliva, along with the reduction in the total amounts of the various salivary constituents such as enzymes, immunoglobulins, and various others. (4). Salivary electrolytes have been known to influence numerous enzymatic processes which are involved in the defence processes within oral cavity such as hyaluronidases, superoxide dysmutases, hystatins and lactof-

Ben-Aryeh i njegovi kolege (6) izvijestili su da je u njihovu istraživanju razina salivarnog kalcija bila povišena, a količina salivarne amilaze snižena i kako nije bilo razlika između mlađih i starijih ispitanika u količini salivarnog natrija, fosfata i magnezija.

Različiti lijekovi mogu smanjiti izlučivanje sline, no rezultati vezani za sijalokemijski nalaz i lijekove su kontroverzni (7,8). Hunter i suradnici(8) istaknuli su da triciklički antidepresivi jako smanjuju izlučivanje sline – posljedica toga je smanjeno izlučivanje natrija, a povećano kalija. To se vjerojatno događa zbog blokade muskarinskih receptora.

Svrha ovog istraživanja bila je odrediti koncentraciju salivarnih analita u sklopu salivarnih enzima koji održavaju integritet oralnih sluznica, a on je oštećen kod bolesnika s OLP-om.

Ispitanici i postupci

Ispitna skupina sastojala se od 25 bolesnika (22 žene i 3 muškarca) s erozivnim OLP-om koji su dolazili na kontrolne preglede u naš Zavod. Izabrani su samo oni s akutnom fazom i kod svakoga je bila prisutna najmanje jedna erozija. Sudionicima je OLP bio patohistološki potvrđen prema kriterijima Svjetske zdravstvene organizacije (9). Prosječna dob oboljelih bila je 73 ±1,4 godine.

Kontrolna skupina sastojala se od 24 zdrava ispitanika (15 žena i 9 muškaraca) u dobi od 24±3,7 godina. Svi su bili znatno mlađi od onih u ispitnoj skupini i prvi smo im put u našem laboratoriju određivali salivarne elektrolite te smo htjeli dobiti referentne vrijednosti.

Uzorke nestimulirane sline dali su između 8 i 12 sati prijepodne, a nisu jeli, žvakali ili pili jedan sat prije uzimanja uzoraka. Slinu su skupljali pet minuta u kalibrirane epruvete (10) i zatim su uzorci držani na -20°C dok nisu analizirani.

Natrij, kalij i klorid određeni su indirektnom potenciometrijom na automatskom biokemijskom uređaju Olympus AU2700 (11). Bakar i magnezij izmjereni su atomskom apsorpcijskom spektrofotometrijom (12), a fosfati kolorimetrijskom metodom uz upotrebu molibdata (13). Ukupni proteini određeni su pirogalol kolorimetrijskom metodom (14). Za određivanje amilaze smo se koristili kontinuiranom kolorimetrijskom metodom (15).

Podaci su analizirani statističkim programom SPSS 10,0 (SPSS Inc. Chicago, Illinois, SAD). Hi-kvadrat test bio je primijenjen kako bi se odredile razlike između spolova i s obzirom na pušenje između ispitivanih skupina. Razlike u dobi i količini izlučene sline dobivene su uz pomoć Mann-Whitneyeva U-testa. Razlike u količini salivarnih analita izračunate su analizom kovarijance. Povezanost između salivarnih analita i količine izlučene sline te broja popušenih cigareta na dan određen je uz pomoć Spearmanove korelaciije.

errin. Decreased salivary protective capacity is of great clinical importance with respect to the oral diseases (5).

Ben-Aryeh et al. (6) reported that salivary calcium levels were increased, salivary amylase was decreased whereas no differences between younger and older participants regarding sodium, phosphate and magnesium could be found.

Various medications might induce decreased salivary secretion, however, the results regarding sialochemistry are controversial (7,8). Hunter et al.(8) reported that tricyclic antidepressants produced a significant reduction in flow and consequent decrease in sodium, but increase in potassium. These effects were presumably due to muscarinic receptor blockade.

The aim of this study was to assess the concentrations of salivary analytes in order to found out whether investigated salivary constituents are disturbed in OLP because most of these electrolytes are part of salivary enzymes which maintain integrity of the oral mucosa which is compromised in OLP patients.

Materials and Methods

Study group consisted of 25 patients (22 females and 3 males) with erosive OLP who came to follow up examinations at our Department. Only patients with active disease (presence of at least one erosion) were selected. All patients had histopatologically confirmed diagnosis of OLP according to the criteria established by WHO (9). Average age of the patient was 73 ±1.4 years.

Control group consisted of 15 females and 9 males who were 24±3.7 years old and who all had healthy oral mucosa and were free of systemic disease. This control group was significantly younger than patient group due to the fact that we did salivary electrolytes for the first time in our laboratory and therefore we sought reference values.

Samples of the whole unstimulated saliva were collected between 8 and 12 AM. Participants were asked to refrain from eating, chewing and drinking at least one hour before sampling. Saliva was collected during 5 minutes into calibrated tubes by expectorating method (10). Saliva samples were kept at -78°C until analysis.

Sodium, potassium and chloride were determined by indirect potentiometry on the automatic biochemical analyser Olympus AU2700 (11). Copper and magnesium were determined by atomic absorption spectrophotometry (12). Phosphates were determined by colorimetric method by use of molybdate (13). Total proteins were determined by pyrogalol colorimetric method (14). Amylase levels were determined by IFCC (continued colorimetric method) (15).

Data were analyzed using SPSS 10.0 (SPSS Inc. Chicago, Illinois, USA) software. Chi square test was used to assess differences in sex and smoking between the groups. Differences in age and salivary flow rate were determined by Mann Whitney U test. Differences in salivary analytes were calculated by analysis of covariance. Relationship between salivary analytes and salivary flow rate as well as number of cigarettes smoked per day was determined by Spearman's correlation.

Rezultati

Demografske karakteristike ispitanika vide se u tablici 1. Oboljeli od OLP-a znatno su stariji u odnosu prema sudionicima iz kontrolne skupine (p=0,0001). Mnogo je više žena imalo OLP odnosu prema muškarcima (p= 0,018), što je u skladu s epidemiološkim karakteristikama te bolesti.

Skupina oboljelih od OLP-a imala je mnogo manju količinu izlučene sline u odnosu prema sudionicima u kontrolnoj skupini (tablica 1). U skupini osoba s OLP-om postojala je velika negativna korelacija između količine izlučene sline i količine salivarnog natrija, kalija, klorida i ukupnih proteina (tablica 2). Nije bilo znatne korelacije između količine izlučene sline i salivarnih analita u kontrolnoj skupini.

Budući da su postojale velike razlike u dobi, spolu i količini sline, ti su čimbenici smatrani posredno povezanima, a

Results

Demographic characteristics of the participants are shown in Table 1. OLP patients were significantly older then controls (p=0.0001). Significantly higher proportion of females was observed among OLP patients (p=0.018) which is in concordance with epidemiological characteristics of the disease.

Patient group had significantly lower salivary flow rate than controls (Table 1). In the patient group significant negative correlation was observed between salivary flow rate and salivary sodium, potassium, chloride and total protein (Table 2). No significant correlation between salivary flow rate and salivary analytes was observed in the control group.

As significant differences in participants' age, sex and salivary flow rate existed these factors were considered as co-

Tablica 1. Demografske karakteristike sudionika u istraživanju
Table 1 Demographic characteristics of the participants

	Pacijenti s OLP-om • Patients with OLP	Kontrole • Controls	p
Dob (srednja vrijednost ± standardna pogreška) • Age (median ± SE)	73 ± 1.4	24 ± 3.7	0.0001*
Spol N (%) • Sex N (%)			
ženski • female	22(88)	15 (62.5)	0.018*
muški • male	3 (12)	9 (37.5)	
Pušenje • Smoking			
Da • yes	2 (8)	9 (37.5)	0.0001*
Ne • no	23 (92)	15 (62.5)	
količina izlučene sline ml/min (srednja vrijednost ± standardna pogreška) • Salivary flow rate ml/min (median ± SE)	0.25 ± 0.05	0.5 ± 0.03	0.0001*

* Značajna razlika (p<0,05) • Significant difference (p<0.05)
OLP - oralni lihen planus • oral lichen planus

Tablica 2. Korelacija između količine izlučene sline i salivarnih analita (Spearmanova korelacija)
Table 2 Correlation between salivary flow rate and salivary analytes (Spearman's correlation)

		fosfat • Phosphate (mmol/L)	natrij • Sodium (mmol/L)	kalij • Potassium (mmol/L)	klorid • Chloride (mmol/L)	bakar • Copper (mmol/L)	magnezij • Magnesium (mmol/L)	amilaza • Amylase (U/L)	ukupni proteini • Total protein (g/L)
Pacijenti s OLP-om • OLP patients	R	-0.282	-0.504*	-0.622*	-0.600*	0.074	-0.113	-0.108	-0.586*
	p	0.171	0.010	0.001	0.002	0.726	0.590	0.608	0.002
Kontrole • Controls	R	0.003	0.130	-0.019	0.377	-0.291	-0.033	-0.162	-0.064
	p	0.989	0.573	0.936	0.092	0.200	0.886	0.482	0.782

* Značajna razlika (p<0,05) • Significant difference (p<0.05)

Tablica 3. Salivarni analiti (srednja vrijednosti ±standardna pogreška) kod pušača i nepušača
Table 3 Salivary analytes (median ±standard error) in smokers and non smokers

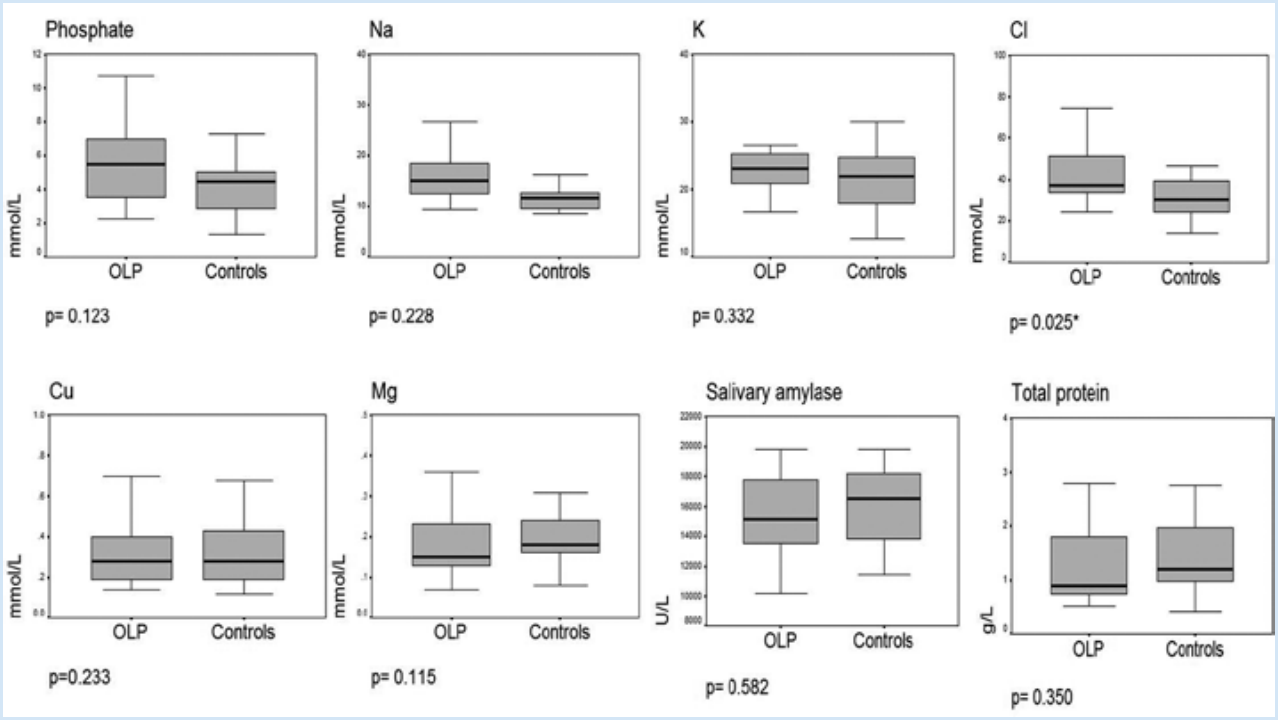
	pušači • Smokers	nepušači • Nonsmokers	p
fosfat • Phosphate (mmol/L)	4.2 ± 0.47	5.31 ± 0.4	0.059
natrij • Sodium (mmol/L)	12.4 ± 1.24	12.7 ± 0.95	0.525
kalij • Potassium (mmol/L)	19.6 ±1	23.1.± 1.33	0.080
kloridi • Chloride (mmol/L)	30.5 ± 5.17	36 ± 2.94	0.284
bakar • Copper (mmol/L)	0.24 ± 0.04	0.29 ± 0.03	0.445
magnezij • Magnesium (mmol/L)	0.18 ± 0.02	0.17 ± 0.02	0.594
amilaza • Amylase (U/L)	15700 ± 792.52	15764 ± 491.6	0.629
ukupni proteini • Total protein (g/L)	1.53 ± 0.19	1.04 ± 0.14	0.509

ne izravno. Obavljena je analiza kovarijance s tim čimbenicima i ustanovljene su znatne razlike u salivarnom kloridu (p= 0,025) (slika 1).

Iako je mnogo više pušača bilo u kontrolnoj skupini (p= 0,0001), pušenje nije bio posredno povezan faktor jer nije bilo razlika u salivarnim analitima između pušača i nepušača (tablica 3). Osim toga, nije bilo korelacije između salivarnih analita i broja popušenih cigareta na dan (tablica 4).

founding. The analysis of covariance with these factors as covariates was performed and significant differences in salivary chloride were observed (p= 0.025) (Figure 1).

Even though significantly higher proportion of smokers (p=0.0001) was observed in the control group, smoking was not considered a cofounding factor because no differences in salivary analytes between smokers and non smokers were observed (Table 3). Furthermore, no correlation between salivary analytes and number of smoked cigarettes per day was observed (Table 4).



*Značajna razlika (p<0,05) • Significant difference (p<0.05)

Slika 1. Koncentracija salivarnih analita kod pacijenata s oralnim lihen planusom i kontrolnih ispitanika (analiza kovarijance)
Figure 1 Concentrations of salivary analytes in oral lichen planus patients and healthy controls (analysis of covariance)

Tablica 4. Korelacija između salivarnih analita i broja cigareta (Spearmanova korelacija)
Table 4 Correlation of salivary analytes and number of cigarettes (Spearman's correlation)

	fosfat • Phosphate (mmol/L)	natrij • Sodium (mmol/L)	kalij • Potassium (mmol/L)	klorid • Chloride (mmol/L)	bakar • Copper (mmol/L)	magnezij • Magnesium (mmol/L)	amilaza • Amylase (U/L)	ukupni proteini • Total protein (g/L)
R	-0.356	-0.256	-0.261	0.095	-0.419	-0.256	-0.025	0.351
p	0.283	0.448	0.439	0.781	0.199	0.448	0.942	0.290

Rasprava

Promijenjene koncentracije salivarnih analita mogu kompromitirati funkcijski kapacitet sline, pa mogu nastati poteškoće u održavanju oralne homeostaze i integriteta sluznice te u zaštiti od stranih proteina i infekcija (5).

Pretpostavili smo da povišene vrijednosti salivarnog kalija, natrija, klorida i proteina mogu biti posljedica pijenja alkohola jer su povišene vrijednosti salivarnih proteina, kalija, natrija i fosfata pronađene kod bolesnika s kroničnim aktivnim hepatitisom kao posljedicom kroničnog alkoholiz-

Discussion

Altered salivary analyte concentrations may compromise the functional capacity of the saliva. Saliva may not be able to play its pivotal part in preserving oral homeostasis, maintenance of mucosal integrity and protection against foreign proteins and infections (5).

We supposed that increased levels of salivary potassium, sodium, and chloride might be due to the drinking habit, as increase in protein, potassium, sodium and phosphate was found in patients having chronic active hepatitis due to the

ma (16). Isto tako smo u našem prijašnjem istraživanju (17) ustanovili povišene vrijednosti natrija i klorida kod bolesnika s oralnim planocelularnim karcinomom. No, ni jedan od ispitanika s OLP-om u ovom istraživanju nije pio alkohol.

Salivarni natrij povišen je bolesnicima sa Sjoegrenovim sindromom (SS-om) i osobama s transplantacijskom bolešću, vjerojatno zbog limfocitne infiltracije žljezdanih kanalića u kojima se resorbira natrij (18). No, samo je dvoje naših ispitanika oboljelih od OLP-a imalo SS.

Vjeruje se da povišenje salivarnog natrija, klorida i fosfata može biti posljedica lijekova koje bolesnici uzimaju. Rezultati objavljenih istraživanja su kontroverzni. Većina istraživača nije ustanovila razlike u sijalokemiji zbog uzimanja lijekova (7, 19). No, van der Berg i njegovi suradnici (20) izvijestili su da bolesnici s kserostomijom potaknutom lijekovima imaju snižene vrijednosti salivarnih analita u odnosu prema zdravim sudionicima u kontroli, i taj je nalaz suprotan rezultatima u ovom istraživanju.

Gandara i suradnici (3) nisu pronašli velike razlike između oboljelih od OLP-a i kontrolne skupine s obzirom na salivarni natrij, kalij, klorid i fosfate te amilazu i ukupne proteine. Njihov nalaz je u suprotnosti s našim rezultatima jer smo kod naših ispitanika s OLP-om ustanovili povišene vrijednosti fosfata, klorida, natrija i ukupnih proteina. Možda su znatne razlike u salivarnim analitima između oboljelih od OLP-a i kontrolnih ispitanika nastale zbog velikih razlika u dobi između ispitne i kontrolne skupine. Naši rezultati u skladu su s rezultatima Naglera i Hershkovicha (2) koji su ustanovili da su salivarni kalij i klorid povišeni kod starijih osoba u usporedbi s mlađima. Isti su autori zaključili kako nije bilo razlika u salivarnim analitima bez obzira na to jesu li ispitanici imali sistemske bolesti/ili su uzimali lijekove. Isti autori zaključili su da su promjene u salivarnim analitima bile izravno vezane za dob, a ne za sistemske bolesti i/ili lijekove koje su ispitanici uzimali.

Smanjeno izlučivanje sline inače je povezano s mnogim promjenama u različitim sastojcima sline (21). Rezultati našeg istraživanja pokazuju da je postojala znatna razlika u količini izlučene sline između bolesnika s OLP-om i kontrolnih ispitanika, tako da su povišeni kalij, natrij, klorid i ukupni proteini posljedica toga, a ne pojačane antimikrobne aktivnosti.

chronic alcoholism (16). Furthermore, in our previous study (17) we found increased salivary levels of sodium and chloride in patients with oral squamous cell carcinoma. However, none of the patients recruited for this study drank alcohol.

Increase in the salivary sodium is seen in patients with Sjögren´s syndrome (SS) and graft-versus-host-disease, probably as a result of lymphocyte infiltration mediated damage to sodium-reapsorbing salivary duct cells (18). However, only two of our patients had disease of the salivary glands, being diagnosed as SS.

We speculated that increase in salivary sodium, chloride and phosphates might be due to the medications that OLP patients take. Results of the published studies regarding sialochemistry due to medication intake are controversial. Most of the authors have not noticed changes in the sialochemistry due to medication intake (7, 19). However, van der Berg et al. (20) reported that patients with drug-induced xerostomia have decreased levels of salivary analytes when compared to the healthy controls a finding which is contrary to our results.

Gandara et al. (3) found no significant differences between OLP patients and controls regarding salivary sodium, potassium, calcium, chloride and phosphate as well as amylase and total proteins. This is in contrast with the results of our study as we found increased levels of phosphate, chloride and sodium in patients with OLP.

It seems that significant differences between patients and controls in salivary potassium and chloride and might be due to the significant age difference between patients with OLP and controls. Furthermore, our results are in concordance with Nagler and Hershkovich (2) who found that salivary potassium and chloride were increased in the older participants when compared to the younger ones. The same authors (2) concluded that there was no difference whether participants had systemic diseases and/or consumed drugs and therefore they concluded that salivary alterations were directly related to age rather than systemic disease and/or drug use.

Last but not least, lowered salivary flow rate has been linked to the changes in the various salivary constituents in other studies (21). We found significant difference between patients with OLP and controls regarding salivary flow rate and one might suppose that increased salivary levels of potassium, sodium and chloride are a results of the difference in salivary flow rate, moreover as salivary proteins were significantly elevated in the OLP group. Therefore, increased salivary potassium, sodium and chloride are not a result of the increased salivary antimicrobial activity in OLP patients as one might think.

Abstract

Objective: It is well known that certain salivary constituents might be disturbed in patients suffering from oral lichen planus (OLP), however, the results of the published studies are inconsistent. The aim of this study was to assess the concentrations of salivary analytes because most of them are part of salivary enzymes which maintain integrity of the oral mucosa which is compromised in OLP patients. **Materials and Methods:** In 25 patients with OLP (73±1.4 yrs) and in the 24 controls (24±3.7yrs) levels of total proteins, amylase, salivary magnesium, calcium, copper, chloride, phosphate, potassium and sodium were determined. Total proteins were determined by pyrogalol colorimetric method. Amylase levels were determined by continued colorimetric method. Salivary sodium, potassium and chloride were determined by indirect potentiometry whereas salivary copper and magnesium were determined by atomic absorption spectrophotometry whereas phosphates were determined by colorimetric method with use of molybdate. Statistical analysis was performed by use of $\chi 2$ test, Mann Whitney U test analysis of covariance and Spearman´s correlation. **Results:** Significantly higher concentration of salivary chloride was detected in OLP patients in comparison to the controls (p=0.025). Furthermore, when the obtained results for salivary analytes were adjusted with respect to the salivary flow rate, increased levels of salivary potassium, sodium, chloride and total proteins were found in patients with OLP when compared to the controls (p=0.622; p=0.504; p=0.600; p=0.586). Concentrations of salivary analytes were not affected by smoking habit. **Conclusions:** Increased levels of salivary sodium, potassium and chloride are probably a result of significant differences in salivary flow rate between patients with OLP and controls and do not indicate increased salivary antimicrobial activity.

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