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Evaluation changes of salivary stress biomarkers after insertion of complete dentures

Анализа маркера стреса у пљувачци код болесника који први пут носе тоталне протезе

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SUMMARY

Introduction/Objective The purpose of this study was to assess the relationships among salivary stress markers salivary α-amylase (sAA) and pH, and patients' stress with new dentures.

Methods An intra-individual evaluation was done, in which 30 participants above 65, both genders, good general health, first time complete dentures (CDs) wearers took part. Measuring of stress biomarkers was done in the unstimulated saliva collected by a so-called spitting method; (1) before any prosthetic treatment, (2) after insertion of a new pair of CDs and (3) two months after CDs delivery.

Results Evaluating the obtained data it was observed that the sAA values are steadily increasing and by the end of the observation period sAA is higher in women but without statistical significance. The pH values increase until the second follow-up period and then it drops. The post-Hoc test showed a statistically significant difference in pH values between 1st vs 2nd ($p = 0.005$) and 2nd vs 3rd ($p = 0.001$) follow-up periods.

Conclusion There was a nonsignificant increase in sAA values during adaptation period. The biggest difference in sAA values between men and women is at the moment of insertion of dentures. The highest pH level is after the insertion of dentures. Since sAA changes are of no statistical significance, the sAA and pH values can't be used as a reliable biomarker in saliva for observing the patients' adaptation, psychological and emotional issues.

Keywords: salivary stress markers; complete dentures; elderly

САЖЕТАК

Увод/Циљ Циљ ове студије је био да се изврши анализа маркера стреса у пљувачци: α-амилазе (сАА) и pH код пацијената који први пут добијају протезе.

Методе Извршена је интраиндивидуална анализа у којој је учествовало 30 болесника старијих од 65 година, оба пола, доброг општег здравственог стања који су први пут добили тоталне протезе (ТП). Мерење маркера стреса извршено је у нестимулисаној пљувачци прикупљеној методом тзв. пљувања и то: (1) пре било какве протетске терапије, (2) после предаје новог пара ТП и (3) 2 месеца након предаје ТП.

Резултати Анализом добијених резултата уочава се стални раст вредности сАА и на крају опсервационог периода сАА је већи код жена, али без статистичке значајности. Вредности pH се повећавају до другог периода праћења и тада опадају. *Post-hoc* тест је показао статистички значајну разлику у вредностима pH између 1.-ог ($p = 0,005$) и 2.-3. периода праћења ($p = 0,001$).

Закључак Током периода адаптације забележен је незнатан пораст вредности сАА. Највећа разлика у вредности сАА између мушкараца и жена је забележен у тренутку предаје нових протеза. Будући да промене сАА немају статистички значај, вредности сАА и pH се не могу користити као поуздан биомаркер у пљувачци за посматрање пацијентовог прилагођавања и постојање психолошких и емоционалних проблема.

Кључне речи: маркери стреса у пљувачци; тотална протеза; старије особе

INTRODUCTION

Implant supported restorations are contemporary treatment options in therapy of complete edentulism. Nevertheless, complete dentures (CDs) are still an alternative teeth replacement option in the elderly, especially in developing countries, mainly due to socio-economic reasons. Hence, with optimal retention and stability, conventional CDs may restore oral functions [1, 2].

The CDs have proven to be beneficial to patients, improving their masticatory efficiency, aesthetics and socio-psychological well-being. Indisputably, conventional CDs are mucosa-borne appliances, thus exerting the occlusal stress on supporting tissues. Also, it is a rigid appliance, introduced in a very dynamic oral environment, and its functionality depends on the patient's ability to coordinate it together with the activity of tongue and masticatory muscles, especially during the unavoidable period of neuromuscular adaptation. Therefore, too many requirements are placed before an edentulous patient in order to overcome the limitations of the appliance such as a rigid CD. Hence, beside the findings that prosthesis retention contributes dramatically to prosthesis acceptance by the patient [3], it may be speculated that successful CDs treatment depends on whether or not it presents a stress to a patient's organism.

Stress may relate to usual transitory problems that arise after the insertion of new CDs, such as discomfort, functional difficulties, sore spots, injured mucosa and different levels of pain, thus extending the period of adaptation [4]. Introducing new CDs may also be a predisposing factor for the onset of salivary changes that affect oral homeostasis and oral mucosal health [4].

Recently, it was summarized that salivary α-amylase (sAA) might be regarded as an indirect indicator of autonomic activation (ANS) expected during psychological stresses [5]. Everyday stress contributes to sAA changes [6, 7] whereas the sAA values increases in response to expectations of medical procedures [8]. In a further investigation it was reported that sAA measurement might be a promising approach for studies of treatment effects [5], as well as a useful marker in the context of pain validation or sleep quality [9, 10]. Together with the most frequently used salivary biomarkers such as cortisol, IgA and sAA for indicating psychologic stress, pH level was recently introduced as a possible useful and non-expensive biomarker [11, 12].

We hypothesized that insertion of new CDs may present an overall stressful environment for patients receiving CDs for the first time. Particularly associated with sensory and motor deterioration in older people and some inevitable level of stress, during the first period of coping with new CDs, may be expected. To our knowledge, there are no existing studies dealing with the impact of new CDs on salivary biomarkers changes in participants first time CDs wearers. Therefore, the aim of the study was to evaluate changes in the levels

of sAA and pH of the unstimulated saliva in the elderly who received their first pair of complete dentures during the neuromuscular adaptation period.

METHODS

Study population

The participants for the study were recruited from edentulous subjects seeking prosthodontic treatment and based on matched inclusion criteria.

The inclusion criteria for the study group (SG) were: 1) subjects over 65, both genders; 2) healthy, without oral and systemic diseases that may influence saliva quality and quantity; 3) first time wearers of CDs without previous experience in wearing mobile appliances.

Individuals with previous experience in wearing dentures, with motor or neurological disorders, and smokers were excluded from the study. Also, the exclusion criterion was using medications that may affect the sympathetic or parasympathetic nervous system.

Total of 30 participants (13 male and 17 female) were allocated to the study group (SG). The participants were instructed not to eat and drink within 2 hours before saliva collection and all were informed on the study procedures and have provided their written consent.

Study design

The study plan included an evaluation of salivary stress biomarkers, sAA and pH, values in the unstimulated saliva collected by the so-called spitting method [13].

SG participants were instructed to seat comfortably, eyes opened, and rinse their mouths for 5s with 5ml of distilled water. Afterwards they were asked to collect their saliva by spitting into a plastic tube every 30s until 5ml of saliva was collected.

Sampling procedure in SG was performed in three investigation steps according to the investigation protocol:

1. Sampling and saliva testing before any prosthetic treatment;

2. Sampling and saliva testing after the insertion of a new pair of CDs; (saliva sampling was performed during the first half of hour, when the CDs were placemed and re-occluded);

3. Sampling and saliva testing two months after the CDs delivery, assuming that the initial neuromuscular adaptation period was finished.

Saliva was taken for all three investigation steps in SG under the same clinical conditions, between 1-2 pm. The study design and data collection methods were approved by the Ethics Committee of School of Dental Medicine, (No: 36/26), and was performed in accordance with the Declaration of Helsinki.

Determination of salivary biomarkers in saliva samples

After sampling, the saliva was not being freezed and markers in it were analiyzed within an hour. All the samples were centrifuged at 3500 rpm for 15 min. During the analysis phase, pH values in saliva were measured, using pH meter (Martini Instruments, USA), calibrated with standard solutions of pH 4.0 and 7.0. The sAA values were determined using colorimetric method and a commercial kit (Alpha-Amylase liquicolor, Human, Germany) following the International Medicine (IFCC) method [14]. The sAA activity of the samples was detected through the enzymatic hydrolysis of the chromogenic substrate 2-chloro-4-nitrophenyl-D-maltotrioside to 2-chloro-nitrophenol (CNP). The rate of increase in absorbance, due to formation of CNP, is measured at 405 nm and is proportional to the sAA activity in the sample. Amylase activity was expressed in units per milliliter (U/ml).

Saliva samples were analyzed at the Laboratory for Biochemistry and Haematology, Faculty of Dental Medicine, University of Belgrade.

Statistical analysis

The data was observed in Statistical Package for Social Science (SPSS software package, version 22.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics for numeric data

were presented by the mean and standard error. Numeric data were analysed using the Mann-Whitney test or the T-test. One-way ANOVA with repeated measures was used for analysis between biomarkers of saliva at three periods (before, after handing over dentures, and after two months). Mixed between-within subject ANOVA was used to evaluate the effect of two factors (time and gender) on biomarkers. Spearman's correlation coefficient was done in order to assess the relationship between the biomarkers od saliva and clinical parameters. P value less than 0.05 was considered statistically significant.

RESULTS

The values of sAA and pH for all patients (male and female) are presented in Table 1. Statistically significant differences were observed between the pH values during the study. A subsequent Post-Hoc test showed a statistically significant difference in pH values between 1st and 2nd follow-up period (before vs after handing over dentures, p=0.005) and between 2nd and 3rd period (after handing over dentures vs after 2 months, p=0.001). sAA values increased during the evaluation period, but this was not statistically significant (Table 1).

The sAA values are slightly higher in women than in men, but with no statistically significant difference. The pH values are similar between the genders and there are no statistically significant differences during the observation period. (Table 2).

The mixed way ANOVA results show a significant time influence on the pH values (p=0.002), while the influence of gender and time together is of no statistial significance. Changes in pH values are similar for both men and women: first we have an increase and then, after the second follow-up period we have a decrease in values. The pH values are higher in men (Figure 1). The influence of time (p=0.703) and time and gender (p=0.679) on the sAA values was not statistically significant, but initially we have lower sAA values in women, which increases during the observation period, while in men we see higher sAA values which reduce over time. The largest difference in sAA values between the genders is immediately after the dentures are handed over (Figure 2).

DISSCUSION

Patients first time CDs wearers are under various factors that influence their adaptation period. Those factors are not fully understood and are addressed as follows: quality of dentures, oral conditions, patient-dentist relationship, attitude toward dentures, patient's personality and socio-economic factors [4]. Mostly, clinical problems are, more or less, easily recognized, but the psychological discomfort during the adaptation period has not been fully explained. This follow-up study assessed the changes of salivary biomarkers, sAA and pH values during the period of neuromuscular adaptation in patients who received their CDs for the first time.

Most of the saliva research focused on saliva's role in digestion, lubricant for food and tongue and microbiology balance of the oral cavity [15, 16, 17]. Furthermore, biomarkers from saliva are reliable, non-invasive and objective, and may be used for monitoring oral health or therapy outcomes [18, 19]. The relevance of saliva to the success of denture retention and oral comfort has been pointed, thus knowledge of the salivary biomarkers further emphasizes the factors that contribute to easier wearing and tolerating CDs [20].

During the adaptation period and the process of learning to use dentures, patients consciously and unconsciously promote more chewing strokes. Thus, when more mastication was needed, significant changes have been observed in the salivary flow rate with consequent changes in amylase and protein concentration, as well as an increase of pH values respectively [21]. In addition to that, another study reported a statistical significance of pH values before and after the CDs insertion [22]. Others, however, have not found significant changes in pH values [4, 23]. Findings of our study are in correlation with previous findings that pH is significantly different before and after dentures insertion [24]. The differences in the reported pH values can also be explained by the different methods used for measurement (pH measuring tapes or electronically).

All patients included in the SG were first time denture wearers and it was expected that they would feel stress related to initial foreign body sensation. However, the findings of the study did not support this hypothesis. Although, the sAA and pH values have changed in time, during the observation period, they were not of statistical significance, especially when sAA values are concerned. Studies show that the sAA values increase when an individual undergoes stress. The sAA value increased within 3 minutes of watching a stressful video, or

5 minutes into a mental arithmetic task. In these situations, the sAA values increase 100% and 200%, while these values lower within 3 minutes of undergoing soothing conditions. Thus, we can explain that dentures don't present great psychological stress for the patient who is exposed to effects of other factors, during such a long observation period, that may have a greater impact [25]. The sAA values higher in men than in women is shown in the research from Matsui et al. [26]. They determined that older men react to denture wearing with a significant increase in sAA values. If we look at the whole adaptation period of two months, we have a steady increase of sAA values in women. Rafeeq [27], arrived to a similar conclusion, however in his research the cortisol level, as a stress biomarker, in women was higher, during the period of adaptation to new dentures, indicating that women are more susceptible to stress.

In our study, that after taking over the new dentures, there is a period of adaptation of the oral cavity, which probably affects the change in the composition of saliva. Therefore, a sense associated with new denture, at the whole adaptation period of two months, may raise level SAA in saliva, but not of statistical significance. This finding might indicate an autonomic body response, while are adjusting to wearing new dentures, because our mind is receiving strong signals from mouth about new dentures.

When pH values were analysed, significant increase was observed among the SG during the observation time, but the opinion is that an increase in pH values is due to enhancement of salivary flow rate accompanied with the insertion of the initial set of CDs [24–29]. Some researchers speculated that CDs in the mouth acted as foreign bodies that simulate saliva flow [30]. However, it is more likely that continuous pressure of CD's base with resultant prolonged the simulation of mucosa mechanoreceptors, that initiates enhanced salivary flow [25, 31]. We are more prone to believe that such documented enhancement of salivary flow influences the salivary composition and the increase of investigated sAA and pH values, rather than psychological stress that CDs patients are exposed to.

According to the psychological models of stress, threat and challenge appraisals have a major effect on the degree of stress experienced by individuals and their emotional reactions to a stressful situation [11]. However, the idea that initiated the study was that patients were exposed to worries about how well they would cope with new the CDs. Whereas worries are more related to appraisals, that constitute more of a challenge than a threat, activating the nervous system in a different way [11], worry dimension rather than emotional dimension is

associated with various physiological markers, such as cardiovascular responses [32]. However, threat appraisals, not challenge ones, are more associated with pH as stress markers [11]. Accepting the before mentioned and summarising the obtained study findings, it is more likely that new CDs present a challenge, not a threat to the organism, as it was not evident, according to stress level markers, that inserting new CDs induced a stress reaction.

In order to analyse only the impact of CDs on saliva stress biomarkers, two main health issues were excluded prior to sampling, as they might influence the objectivity of the results. Participants of both groups were non-smoking individuals, due to reported statement that smoking increases stress hormone levels with associated changes in pH [33]. Also, the participants were instructed to seat calmly restraining from any physical activity prior the sampling, since firm association between physical activity and stress levels exists.

Although mounted as a pilot study, the main limitation is the relatively small number of participants with reflective lack of possible generalization of the findings. We did our best to keep the SG as homogenous as possible, dentures were constructed by the same dentist and dental technician, using materials from the same manufacturer with satisfactory retention and stabilisation. Moreover, since it is well known that sAA and salivary pH values change throughout the day, variance of daily values and subsequent influence on the findings may be another limitation.

CONCLUSION

During the adaptation period to new CDs the sAA values are increased. The changes in sAA values in regard to the participant's gender shows that sAA values in women are increasing over time while the sAA values in men are decreasing. The study shows that the highest pH value is immediately after the dentures are delivered. Since sAA changes are of no statistical significance, sAA and pH values can't be used as reliable biomarkers in saliva, for monitoring the patient's adaptation, psychological and emotional issues.

Conflict of interest: None declared.

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Table 1. Monitoring of stress factors (salivary α-amylase and pH) in all participants during all study periods

Biochemical parameters Mean (SE)	Evaluation period			p	
	Before	After handing over dentures	After 2 mos.		
Salivary α-amylase	264.0 (49.9)	290.0 (50.9)	318.0 (44.3)	0.680[a]	
pH	7.3 (0.5)	7.7 (0.1)	7.1 (0.1)	0.001*[a]	1 *vs*. 2, p = 0.005*[b] 2 *vs*. 3, p = 0.001* 1 *vs*. 3, p = 0.119

[a]One-way Anova with repeated measures;
[b]Post Hoc analysis: Bonferroni test;
*statistically significant

Table 2. Comparison values of salivary α-amylase and pH between sexes during the study period

Variables Mean (SE)	Male	Female	p
Age	66 (1.3)	65 (0.9)	0.837
Salivary α-amylase			
Before	269 (66.7)	241.3 (54.5)	0.752
After handing over dentures	295.2 (62.6)	252.7 (66.1)	0.652
After 2 mos.	300.3 (54.1)	332 (68.4)	0.728
pH			
Before	7.2 (0.2)	7.4 (0.1)	0.382
After handing over dentures	7.6 (0.2)	7.8 (0.1)	0.327
After 2 mos.	7.1 (0.1)	7.1 (0.1)	0.686

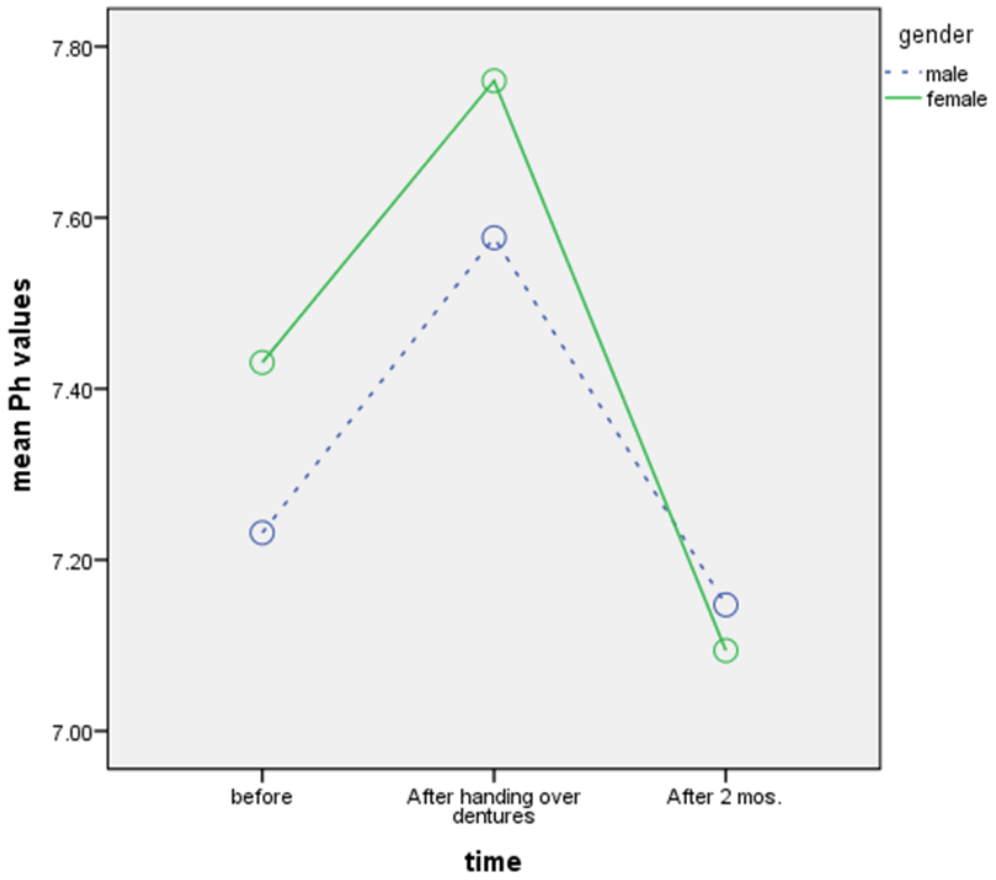

Figure 1. Effects from two factors (time and gender) on pH levels

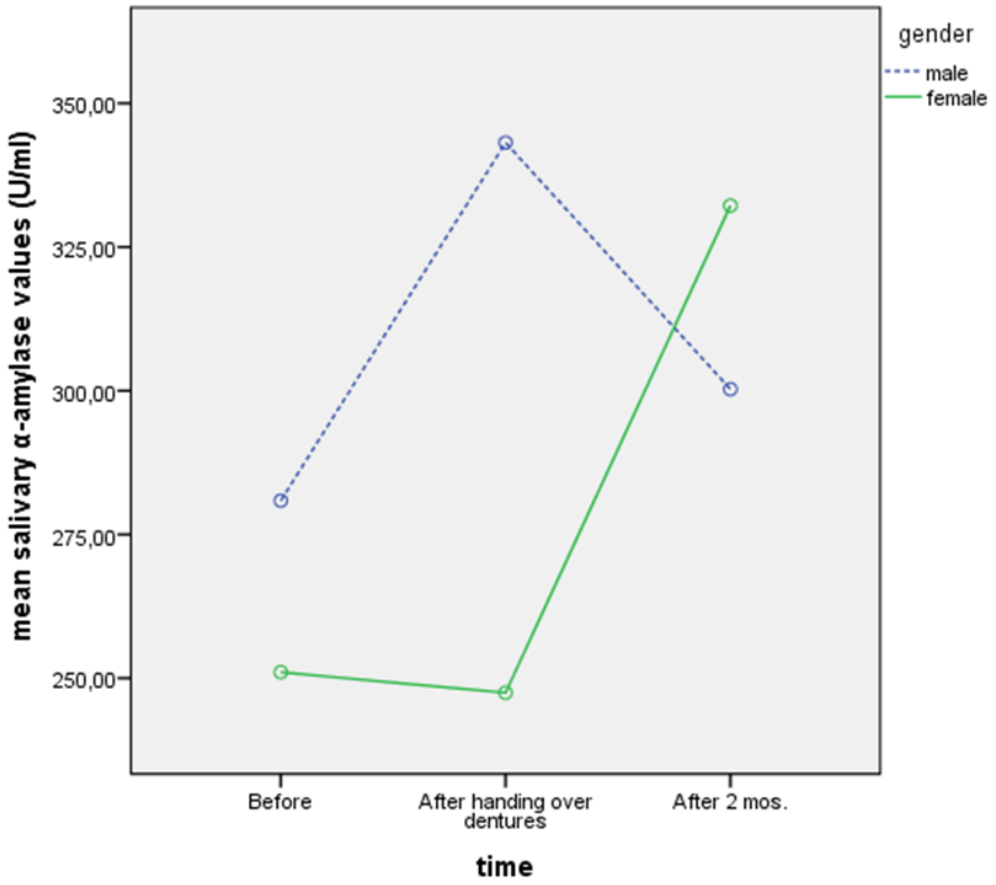

Figure 2. Effects of two factors (time and gender) on salivary α-amylase levels

