ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Evaluation of salivary stress biomarker changes after the insertion of complete dentures

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SUMMARY

Introduction/Objective The purpose of this study was to assess the relationships between 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 older than 65 years, both sexes, in good general health, first-time complete dentures (CDs) wearers, took part. Measuring of stress biomarkers was done in the unstimulated saliva collected by the 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 Upon evaluating the obtained data it was observed that the sAA values steadily increased and by the end of the observation period sAA was higher in women but without statistical significance. The pH values increased until the second follow-up period, after which they dropped. The post-hoc test showed a statistically significant difference in pH values between the first *vs*. the second (p = 0.005) and the second *vs*. the third (p = 0.001) follow-up periods.

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

Keywords: salivary stress markers; complete dentures; elderly

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].

CDs have proven to be beneficial to patients, improving their masticatory efficiency, esthetics, 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 the tongue and the masticatory muscles, especially during the unavoidable period of neuromuscular adaptation. Therefore, many requirements are placed before an edentulous patient in order to overcome the limitations of an 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 expected during psychological stresses [5]. Everyday stress contributes to sAA changes, whereas the sAA values increase in response to expectations of medical procedures [6, 7, 8]. In a further investigation it was reported that sAA measurement might be a promising approach for studies of treatment effects, as well as a useful marker in the context of pain validation or sleep quality [5, 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 the 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, some inevitable level of stress may be expected during the first period of coping with new CDs. To our

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knowledge, there are no existing studies dealing with the impact of new CDs on changes in salivary biomarker levels in first-time CDs wearer participants. Therefore, the aim of the study was to evaluate the 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 as follows: 1) subjects over 65 years of age, both sexes; 2) healthy, without oral and systemic diseases that may influence the saliva quality and quantity; 3) first-time wearers of CDs without previous experience in wearing mobile appliances.

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

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

Study design

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

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

Sampling procedure in the 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 an hour, when the CDs were placed and re-occluded);
3. Sampling and saliva testing two months after the CDs delivery, assuming that the initial neuromuscular adaptation period was finished.

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

Determination of salivary biomarkers in saliva samples

After the sampling, the saliva was not frozen and the markers in it were analyzed within an hour. All the samples were centrifuged at 3500 rpm for 15 minutes. During the analysis phase, pH values in saliva were measured, using pH meter (Martini Instruments, USA), calibrated with standard solutions with a pH of 4 and 7, respectively. The sAA values were determined using the colorimetric method and a commercial kit (Alpha-Amylase LiquiColor, Human, Wiesbaden, Germany) following the International Federation of Clinical Chemistry and Laboratory Medicine 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 the 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, School of Dental Medicine, University of Belgrade.

Statistical analysis

The data was analyzed in IBM SPSS Statistics, Version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics for numeric data were presented by the mean and standard error. The numeric data were analyzed using the Mann–Whitney test or the t-test. One-way ANOVA with repeated measures was used for the analysis between the biomarkers of saliva at three periods (before, after handing over the dentures, and after two months). Mixed between-within subject ANOVA was used to evaluate the effect of two factors (time and sex) on the biomarkers. Spearman's correlation coefficient was done in order to assess the relationship between the saliva biomarkers and clinical parameters. A 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 the first and the second follow-up period (before *vs.* after handing over the dentures, p = 0.005) and between the second and the third period (after handing over dentures *vs.* after two months, p = 0.001). The sAA values increased during the evaluation period, but this was not statistically significant (Table 1).

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

The mixed-design ANOVA results show a significant time influence on the pH values (p = 0.002), while the influence

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 months		
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 months	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 months	7.1 (0.1)	7.1 (0.1)	0.686

of sex and time combined is of no statistical significance. Changes in pH values were similar for both men and women: initially we have an increase, followed by a decrease in values after the second follow-up period. The pH values are higher in men (Figure 1). The influence of time (p = 0.703) and time and sex (p = 0.679) on the sAA values was not statistically significant, but initially there were lower sAA values in women, which increased during the observation period, while in men there were higher sAA values which reduced over time. The largest difference in sAA values between the sexes was immediately after the dentures were handed over (Figure 2).

DISSCUSION

First-time CDs wearers are under various factors that influence their adaptation period. These factors are not fully understood and are addressed as follows: the quality of dentures, oral conditions, patient–dentist relationship, attitude toward dentures, patient's personality, and socio-economic factors [4]. Clinical problems are mostly 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 the saliva's role in digestion, as a lubricant for food and tongue, and in microbiological 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 for the success of denture retention and oral comfort has been underlined; thus, the knowledge of the salivary biomarkers further emphasizes the factors that contribute to CDs being utilized more comfortably and easily [20].
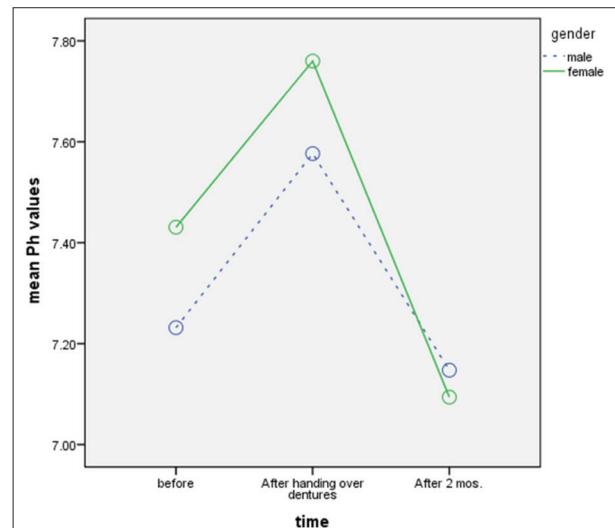
Figure 1. Effects from two factors (time and sex) on pH levels
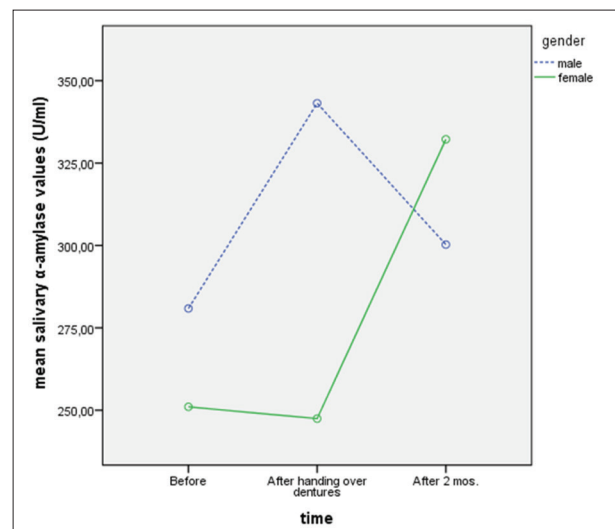
Figure 2. Effects of two factors (time and sex) on salivary α-amylase levels

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 in an increase of pH values [21]. In addition to that, a study reported a statistical significance of pH values before and after the insertion of CDs [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 the measurements (pH-measuring tapes or electronically).

All the patients included in the SG were first-time denture wearers and it was expected that they would feel stress related to the initial foreign body sensation. However, the findings of the study did not support this hypothesis. Although the sAA and pH values changed during the observation period, they were not of statistical significance, especially in regard to sAA values. Studies show that the sAA values increase when an individual undergoes stress. The sAA value increases within three minutes of watching a stressful video, or five minutes into a mental arithmetic task. In these situations, the sAA values increase 100% and 200%, respectively, while these values lower within three minutes of undergoing soothing conditions. Thus, we can explain that dentures do not 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 are shown in the research by 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 at a similar conclusion; however, in his research, the cortisol level, as a stress biomarker, was higher in women during the period of adaptation to new dentures, indicating that women are more susceptible to stress.

In our study, there is a period of adaptation of the oral cavity to the new dentures, which probably affects the composition of the saliva. Therefore, the sense associated with new dentures during the whole adaptation period of two months may increase the sAA level in saliva, but not enough to be of statistical significance. This finding might indicate an autonomic response of the body as it adjusts to wearing new dentures, because our mind is receiving strong signals from the mouth that new dentures are present.

When pH values were analyzed, a significant increase was observed in the SG during the observation period, but the opinion is that an increase in pH values was due to the enhancement of salivary flow rate accompanied by 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 prolonged the stimulation of mucosa mechanoreceptors, that initiates enhanced salivary flow [25, 31]. We are more prone to believe that such an enhancement of salivary flow, rather than psychological stress that CDs patients are exposed to, influences the salivary composition and the increase of investigated sAA and pH values.

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 study was initiated by the idea that patients were exposed to worries about how well they would cope with new CDs. Whereas worries are more related to appraisals, which 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 aforementioned and summarizing 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 analyze only the impact of CDs on saliva stress biomarkers, two main health issues were excluded prior to the sampling, as they might have influenced the objectivity of the results. Participants of both groups were non-smoking individuals, due to a reported statement that smoking increases the stress hormone levels, with associated changes in pH [33]. Also, the participants were instructed to sit calmly, restraining from any physical activity prior to the sampling, since there is a firm association between physical activity and stress levels.

Although designed as a pilot study, the main limitation is the relatively small number of participants with a reflective lack of a possible generalization of the findings. We did our best to keep the SG as homogenous as possible; the dentures were constructed by the same dentist and dental technician, using materials from the same manufacturer with satisfactory retention and stabilization. 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

The sAA values are increased during the adaptation period to new CDs. The changes in sAA values in regard to the participant's sex show that sAA values in women increase over time, while the sAA values in men decrease. The study shows that the highest pH value occurs immediately after the dentures are delivered. Since sAA changes are of no statistical significance, sAA and pH values cannot 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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Анализа маркера стреса у пљувачки код пацијената који први пут носе тоталне протезе

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САЖЕТАК
Увод/Циљ Циљ ове студије је био да се изврши анализа маркера стреса у пљувачки 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. Мерење маркера стреса извршено је у нестимулисаној пљувачки прикупљеној методом тзв. пљувања и то: (1) пре било какве протетске терапије, (2) после предаје новог пара тоталних протеза и (3) два месеца после предаје тоталних протеза.
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 првог и другог (*p* = 0,005) и другог и трећег (*p* = 0,001) периода праћења.
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 пљувачки за посматрање болесниковог прилагођавања и постојање психолошких и емоционалних проблема.
Кључне речи: маркери стреса у пљувачки; тотална протеза; старије особе