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 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 prosthetic 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
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 changes after the insertion of complete dentures.

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 saliva samples.

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

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-nitropheryl-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 salivary 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
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).

**DISCUSSION**

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].

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

| Biochemical parameters | Evaluation period | p   |
|------------------------|-------------------|-----|
|                        | Mean (SE)         |     |
| Salivary α-amylase     | Before            | 264.0 (49.9) |
|                        | After handing over dentures | 290.0 (50.9) |
|                        | After 2 months    | 318.0 (44.3) |
| pH                     | Before            | 7.3 (0.5) |
|                        | After handing over dentures | 7.7 (0.1) |
|                        | After 2 months    | 7.1 (0.1) |

*p-value: 1 vs. 2, p = 0.005*<sup>b</sup> 2 vs. 3, p = 0.001* <sup>a</sup> 1 vs. 3, p = 0.119

*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 |

**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; how-
ever, 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 com-
position 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 en-
hancement 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 simu-
late saliva flow [30]. However, it is more likely that continu-
ous pressure of CD’s prolonged the stimulation of mucosa
mechano receptors, 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 re-
actions 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 ap-
praisals, 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 organ-
ism, 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 associ-
ated changes in pH [33]. Also, the participants were in-
structed to sit calmly, restraining from any physical activ-
ity 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 satisfac-
tory 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 маркерна 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 маркерна стреса у пљувачки-амилазе (сАА) и рН код пацијената који први пут добијају протезе.

Методе. Извршена је интраиндивидуална анализа у којој је искључен утицај предаје нових протеза. Анализа маркерних спрема у пљувачкој врсту суграђана који први пут добијају протезе показала је значајан увећај рН и сАА код жена у тренутку предаје нових протеза у отприликт постојању психолошких и емоционалних проблема.

Закључак. Резултати анализа код пацијената који први пут носе тоталне протезе показаху да је већи код жена, али без статистичког значаја. Вредности рН и сАА код жена су повећане у тренутку предаје нових протеза у поређењу са унутрашњим рН и сАА код жена који носе протезе. Откривена је значајан увећај рН код жена у тренутку предаје нових протеза у поређењу са унутрашњим рН и сАА код жена који носе протезе.

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