European Review for Medical and Pharmacological Sciences 2018; 22: 1191-1195

Evaluation of salivary surface tension in a cohort of young healthy adults

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Abstract. – OBJECTIVE: To determine salivary pH, flow rate (FR) and surface tension (γ_s) in a cohort of 30 healthy young adults. To acquire cohort biological independent variables (age, gender, weight, height, medications, smoking, pathologies, and allergies) and to correlate them with pH, FR and γ_s obtained values. Evaluate the possible variation of the γ_s values during the time after the withdrawal and the influence of the operational abilities of the experimenting operators. Evaluate the relationship between γ_s, pH and FR and the dependence between pH and FR.

PATIENTS AND METHODS: Non-stimulated saliva samples were taken in four different time span, for three days, with a drooling method for 15 minutes. The saliva sample was analyzed, in terms of γ_s, by two different operators (OP1 and OP2), twice consecutive (γ_s-1 and γ_s-2) for a total of 360 measurements. The γ_s was calculated using the du Noüy method. The FR was evaluated by weighing technique and pH by pH indicator papers.

RESULTS: The measurements of γ_s performed by two different operators (OP1, OP2) showed respectively average values of 46.46 mN/m and 43.45 mN/m, while the mean FR was 0.29 ± 0.13 mL/min and the average pH was 7.1 ± 0.43. There were no significant correlations between γs and the biological variables analyzed.

CONCLUSIONS: We can consider as reference values, in a sample of young adults, γ_s 45.56 ± 6.51 mN/m.

Key Words:
Saliva, Surface tension, Salivary pH, Salivary flow rate.

Introduction

The surface tension of a liquid (γ) is the resultant of the forces acting on the liquid surface, allowing the liquid to minimize the surface exposed to the environment reducing its interactions with it. In other words, the surface tension is the ratio between the force required to increase the surface of the liquid (W) and the area where the force is applied (A) $\gamma = W / A$. The unit of measurement of γ is $mN \cdot m^{-1}$ (milliNewton/meter).

The superficial salivary tension (γ_s) is the measure of its ability to spread into the oral cavity and, consequently, to form a protective biofilm on the mucous membrane. A low value of γ_s indicates the presence of strong interactions between the oral mucosa and the biological fluid, which allow full coat of the oral cavity by the saliva. According to some studies, very high or low values of γ_s affect the adhesion of the bacterial plaque to the dental surfaces[1,2]. In addition, γ_s is considered an important feature for the retention of total prostheses. Indeed, as mentioned above, one of the consequences of the liquids surface tension is the tendency to minimize the free surface area, generating curved surfaces such as raindrops or liquid meniscus. The presence of a curved surface creates a pressure difference on that surface and, if the curvature is negative, as in the case of liquids between two surfaces, the pressure is negative. Such negative pressure applies a force that tends to attract the two surfaces. This also happens in the case of thin saliva film placed between the oral mucosa and the base of the prosthesis, generating a retention force that can increase the prosthesis stability[3-5].

γ_s determination may be useful for the development of new diagnostic methods in dentistry; according to some studies, in children with caries, their saliva showed a 29% higher rate of γ_s compared to that of children without caries[6].

Measuring γ_s could help medical doctors to identify obstructive sleep apnea; indeed, the reduction of γ_s may decrease the mucosal adhesion forces and increase the accessibility of the airways[7,8]. From a literature review appears that high saliva γ_s reduces the particles wettability

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of pharmaceutical drugs slowing the dissolution thereof[9]. Different methodologies for the γ_s measurement are used and the obtained results are not comparable to each other. Moreover, it is prior art that monitoring salivary flow rate (FR) and pH is of primary importance in dentistry; indeed, it has been shown that alteration of these values reduces orodental health[6,10].

Thus, our research has focused on the determination of γ_s and its relationship with pH and FR.

The aim of the work was to determine pH, FR, and γ of non-stimulated saliva (U.W.S.) in a cohort of healthy young adults. Investigate the biological variables of the cohort (age, gender, weight, height, medications, tobacco, pathologies, allergies), correlating among pH, FR, and γ values.

Evaluate the possible variation of γ_s values after withdrawal, the differences between samples taken at different times throughout the day and the reproducibility of the measure of γ_s by the operators involved in its determination. Evaluate any relationship between the three variables γs, pH, and FR.

Patients and Methods

Saliva was sampled by 30 volunteers, healthy young adults, 21 women (mean age 22.6 years) and 9 men (mean age 21.6 years). Subjects, students of Dental Hygiene of the University of Eastern Piedmont expressed their informed consent to be subjected to anamnestic and oral examination, previously approved by Ethics Committee (Protocol 340lCL, Study No. CE 56/10). Each subject was asked to follow specific behavioral rules from the day before the drawing.

U.W.S. was collected by a drooling method for 15 minutes, in controlled temperature and humidity (25°C – 60%)[11-13].

Each subject was subjected to saliva sampling on three different working days, not necessarily consecutive, in which four withdrawals were made in four different time ranges (09.00-10.30, 10.30-11.30, 11.30-13.00, 13.30-15.30).

The FR was evaluated by weighing technique, pH with litmus papers (Saliva-Check BUFFER, GC America, measuring range 5.0-7.8) and γ with Sigma 703D tensiometer (KSV Instruments Inc. USA) equipped with a platinum ring (du Noüy's method). Two consecutive measurements were performed (γs-1 and γs-2) on a total of 360 measurements performed by two different operators (OP1 and OP2, which generated 49 and 51% of the analyses respectively). Operators have previously been trained for both sampling methods and pH, FR and γ_s measurements.

During measurement of γ_s, attention was paid in placing correctly the ring (complete immersion in the liquid to be analyzed and absence of foam on the sample surface). Saliva samples were gradually transferred from the sample tube to the measuring container using a pipette (in high-density polyethylene), the ring was immersed in the sample and, then, extracted from the liquid at a constant speed. The instrument measures the pull force needed to extract the ring from the liquid: this is displayed on the instrument display and corresponds to the value of γ.

Statistical Analysis

A descriptive statistic on the collected values of pH, FR, and γ_s were made, based on: mean, standard deviation, relative standard deviation. The values of the collected γ_s were divided by the operator variables (OP1 and OP2) and the order of measurement (γ_s-1 and γ_s-2), these groups averages were compared by Student's test; in case of data distribution was not normal, the logarithmic transformation of the values was performed. A correlation table was also developed between all available variables, previously normalized, (both experimental and descriptive), determining the coefficient of correlation R. A $p \leq 0.05$ was considered statistically significant. To analyze the data Microsoft® Excel 365 software was used.

Results and Discussion

Four subjects were excluded from the calculation of descriptive analysis of pH, FR and γ_s values. They were subjects with chronic illnesses, in particular: one subject with gastric reflux, one with celiac disease and two with asthma, were found in the cohort. In addition, 8 subjects reported having seasonal respiratory allergies. However, mild degree and/or insufficient recurrence cases were considered to exclude subjects from experimentation.

The average value of FR was 0.29 ± 0.13 mL/min and the pH was 7.10 ± 0.43. The mean values of γ_s obtained from the two subsequent measurements on the same sample were γ_s -1 = 45.56 ± 6.51 mN m and γ_s-2 = 43.82 ± 8.41 mN m, the difference between the two groups of measurements were not statistically significant. These values are slightly lower than the values found in the litera-

ture (57.7 ± 4.5, 57.0 ± 2.0, 53.1 ± 2.7 and 58.98 ± 2.18)[1,8,14,15]. This may be explained by the different measurement techniques used, or by the different measurement conditions (e.g., temperature) or mathematical corrections of γ values or the selection methods of the cohort[1,6-9,14,15].

In this study, with the aim of evaluating the possible influence on γ_s by the subjects descriptors, a correlation analysis was performed by investigating the possible dependence of surface tension on some physical and physiopathological features (variables 1-9) of subjects selected for the study or other measurements evaluated during the study (FR and pH). The results of the analysis are shown in Table I. As can be seen, the values of the calculated correlation coefficient (R) are always very low (Table I). The R values for γ_s-1 with variables 1-10 is less than 0.1 excepted for the correlation with the weight of subjects (R = 0.183). For γ_s-2, R values are slightly higher than 0.1 with gender variables (R = 0.153), weight (R = 0.206) and height (R = 0.189). Therefore, the results suggest that the physical characteristics of donor subjects (variables 1-4) do not have a significant influence on the salivary surface tension value also, the possible intake of drugs (for seasonal or contraceptive affections) or pathologies do not significantly change the value of γ_s. It is interesting to note that the values of γ_s-1 and γ_s-2 are only partially dependent on each other (R = 0.42), indicating that the values of γ_s, obtained by consecutively measuring the same sample, are partially correlated; the mean values of γ_s-1 and γ_s-2 are not significantly different between them and the differences between the two measures fall within the limits of the experimental error. Another important aspect is that the values of γ_s showed no correlation with the pH of the sample (R = 0.006 for γ_s-1 and 0.026 for γ_s-2). Also, correlation with FR is low (R 0.17 for γ_s-1 and 0.20 for γ_s-2 respectively). This result suggests that the surface tension value does not depend on the pH of the saliva and is possible minimally related to the saliva production rate. Regarding pH and FR and their correlation with the variables considered, both showed a partial dependency on allergies, with R values of 0.247 and 0.220, respectively, indicating that their values could be altered in the presence of these pathologies, but their reduced severity and recurrence of clinical manifestations in the observed cases does not allow for certain and significant conclusions. The correlation analysis between FR and pH (R = 0.523) confirmed the findings of previous works[11-13,16]. The pH of the saliva varies in part depending on the production rate of this biological fluid, i.e., FR such correlation was associated with an increase in the concentration of bicarbonate and salivary proteins, which are dependent on the salivary secretion rate[11-13,16].

The measurements performed by the two operators OP1 and OP2 showed respectively mean values of γ_s-1 equal to 44.48 ± 10.52 mN/m and 43.02 ± 10.80 mN/m. These values are not significantly different (p>0.05). Similarly, median values (OP1 = 45.72 mN/m and OP2 = 44.08 mN/m) appear to be substantially similar, while a greater difference is observed the mode values obtained by the two operators (OP1 = 51.39 mN/m and OP2 = 43.54 mN/m).

The values of γ_s in the range of 30-60 mN/m obtained by the two operators were grouped into 6 classes with a range of 5 mN/m (Class I 30-35, Class II 35-40, Class III 40-45, Class IV 45-50, Class V 50-55, Class VI 55-60). The frequencies for the classes obtained for OP2 (I-20, II-31, III-27, IV-27, V-35, VI-21) are more homogeneous than those obtained for OP1 (I-13, II-13, III-34, IV-29, V-36, VI-27), and OP1 low γ_s classes (I and II) have lower frequencies. This probably indicates that, since during the measurement the ring extraction was made manually by the operator, OP1 seems to have less sensitivity and less accuracy when performing the measurement. This indication is confirmed by data analysis performed on the complete set of data and considering the average values of γ_s in the individual measurement days obtained by the 2 operators (Figure 1). Again, in the case of OP2, there is a slight fluctuation of γ_s values over the 13-day measurement compared

Table I. Correlation coefficients from statistical analysis.

		Correlation Coefficient – R			
Var. Nr.	**Variables**	**γ_s-1**	**γ_s-2**	**FR**	**pH**
1	Age	0.008	0.010	0.005	0.148
2	Gender	0.085	**0.153**	0.261	0.271
3	Weight	**0.183**	**0.206**	0.365	0.249
4	Height	0.076	**0.189**	0.294	0.392
5	Drugs	0.018	0.020	0.072	0.088
6	Contraceptives	0.077	0.077	0.037	0.022
7	Smoking	0.009	0.073	0.088	0.241
8	Pathologies	0.078	0.042	0.034	0.030
9	Allergies	0.079	0.086	**0.220**	**0.247**
10	Weather	0.009	0.004	0.176	0.057
	γs-1	1.00	**0.421**	0.168	0.006
	γs-2	0.421	1.00	**0.200**	0.026
	FR	0.168	0.200	1.00	0.523
	pH	0.006	0.026	**0.523**	1.00

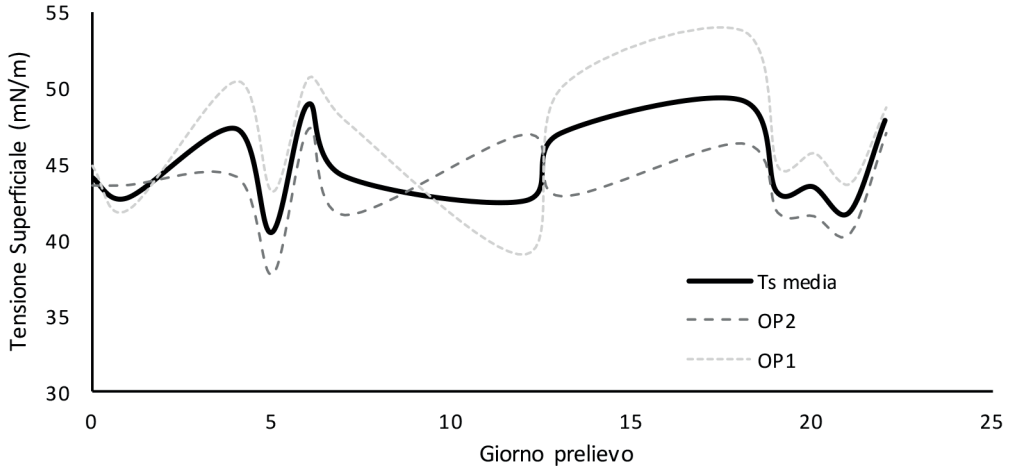

Figure 1. Influece of operators on surface tension measurements on withdrawal days.

to what was observed for OP1. Moreover, for both OPs there are no significant trend variations, i.e., always the tendency of OP1 to be positioned with higher γ_s values than the average. The variation of γ_s mean values during different withdrawal times is not significant due to high standard deviation values holding average values into the confidence interval. Nevertheless, the variability of the biological sample does not allow to observe significant variations between the two operators.

Conclusions

In this study a homogeneous sample was analyzed, which is shown in the mean values of γ_s-1 and γ_s-2, respectively 45.56 ± 6.51 mN/m and 43.82 ± 8.41 mN/m, respectively in which standard deviations are not substantially different. However, in some cases, there was considerable variability between first and second measurement, for which a rational explanation could not be given. Therefore, reference values of a young healthy adult sample can only be considered as γ_s-1 values. This study allows to generate a dataset for a possible comparison with a cohort of subjects with different characteristics than those considered, thus allowing to compare saliva values of patients with some pathological conditions and/or oral lesions with this values, considered to be physiological ones.

Our analysis also confirmed the correlation between pH and FR as in previous studies[11-13,16]; however, differences in the variations of the mean values of γ_s over time, and in function of the pH and F.R variables, have not been founded.

Differences in γ_s values are mainly attributable to the biological variability of the sample and, to a lesser extent, to the sensitivity of individual operators in the acquisition of measurements. However, it is considered necessary to create a protocol for saliva collecting and measuring γ_s, which is important for U.W.S. study and its clinical applications for diagnostic and therapeutic purposes.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Glantz PO. The surface tension of saliva. Odontol Revy 1970; 21: 119-127.
2) Christersson CE, Dunford RG, Glantz PO, Baier RE. Effect of critical surface tension on retention of oral microorganisms. Scand J Dent Res 1989; 97: 247-256.
3) Barbenel JC. Physical retention of complete dentures. J Prosthet Dent 1971; 26: 592-600.
4) Darvell W, Clark RKF. The Physical mechanisms of complete denture retention. Br Dent J 2000; 189: 248-252.
5) Gibert Y, Soulet H, Cariou JM, Collette G. Role of surface tension and viscosity of saliva in complete removable prosthetics. Bull Group Int Rech Sci Stomatol Odontol 1977; 20: 165-187.
6) Kazakov VN, Udod AA, Zynkovych II, Fainerman VB, Miller R. Dynamic surface tension of saliva: general relationships and application in medical diagnostics. Colloids Surf B Biointerfaces 2009; 74: 457-461.
7) Kirkness JP, Christenson HK, Wheatley JR, Amis TC. Application of the 'pull-of' force method for mea-

surement of surface tension of upper airway mucosal lining liquid. Physiol Meas 2005; 26: 677-688.

8) Kaway M, Kirkness JP, Yamamura S, Imaizumi K, Yoshimine H, Oi K, Ayuse T. Increased phosphatidylcholine concentration in saliva reduces surface tension and improves airway patency in obstructive sleep apnoea. J Oral Rehabil 2013; 40: 758-766

9) Gittings S, Turnbull N, Henry B, Roberts CJ, Gershkovich P. Characterisation of human saliva as platform for oral dissolution medium development. Eur J Pharm Biopharm 2015; 91: 16-24.

10) Foglio Bonda PL, Brilli K, Pattarino F, Foglio Bonda A. Salivary flow rate and pH in patients with oral pathologies. Eur Rev Med Pharmacol Sci 2017; 21: 369-374.

11) Foglio Bonda PL, Migliario M, Rocchetti V, Pattarino F, Foglio-Bonda A. Daily and annually variation of unstimulated whole saliva flow rate and pH and their relation with body profile in healthy young adults. Eur Rev Med Pharmacol Sci 2013; 17: 2538-2545.

12) Foglio Bonda A, Pattarino F, Foglio Bonda PL. Kinematic viscosity of unstimulated whole saliva in healthy young adults. Eur Rev Med Pharmacol Sci 2014; 18: 2988-2994.

13) Fenoll-Palomares C, Munoz-Montagud JV, Sanchiz V, Herreros B, Hernández V, Mínguez M, Benages A. Unstimulated salivary flow rate, pH and buffer capacity of saliva in healthy volunteers. Rev Esp Enferm Dig 2004; 96: 773-783.

14) Kirkness JP, Amis TC, Wheatley JR. Determining the surface tension of microliter amounts of liquid. J Colloid Interface Sci 2000; 232: 408-409.

15) Braddock L I, Margallo E, Barbero GJ. Salivary surface tension and turbidity in cystic fibrosis. J Dent Res 1970; 49: 464.

16) Wu K, Ke JY, Chung CY, Chen CL, Hwang TL, Chou MY, Wong AM, Hu CF, Lee YC. Relationship between unstimulated salivary flow rate and saliva composition of healty children in Taiwan. Chang Gung Med J 2008; 31: 281-286.