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Salivary pH and flow rate in menopausal women

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Abstract. – OBJECTIVE: This study aims at determining pH and Flow Rate (FR) of Unstimulated Whole Saliva (UWS) in a sample of 120 ♀ (60 menopausal women and 60 healthy fertile women with similar mean age); detecting the DMFT index (Decayed, Missing, Filled Teeth index) and evaluating any correlations between pH, FR, age and DMFT.

PATIENTS AND METHODS: Concerning the day before sample collection, patients were advised to keep a relaxed attitude and not to practice sports. They were also told to not eat or drink during the hour preceding sampling procedures. Saliva was collected via "spitting" method. Each sampling session started at 11:00 a.m., lasted for 5 minutes and used a pre-weighed, dry, deionized and sterile test tube. The procedure took place under controlled environmental temperature and humidity conditions (means 23.27°C; 60.08%). FR was evaluated via weighing technique and pH was measured with a portable pH-meter.

RESULTS: There was a minimal but significant pH difference (0.11; $p<0.05$) between menopausal women (6.75 ± 0.34) and fertile women (6.86 ± 0.24); and a FR difference (0.19; $p<0.0001$) between menopausal women (0.29 ± 0.17 mL/min) and fertile women (0.48 ± 0.19 mL/min). Correlation (R2) between pH and age was 0.0135 for fertile women and 0.0055 for menopausal women; while the correlation between FR and age was 0.0673 for fertile women and 0.139 for menopausal women. Mean DMFT was 11.93 ± 7.14 in menopausal women and 12.23 ± 6.37 in fertile women.

CONCLUSIONS: We observed a minimal decrease in pH and a decrease in FR in menopausal women. Further studies will be needed to investigate the possible role of other environmental and individual variables in the determination of such values.

Key Words:
Human saliva, Salivary pH and flow rate, Menopause.

Introduction

The generic term "saliva" refers to the liquid produced by major and minor salivary glands attached to the oral cavity[1-6]. Its components can be categorized as "duct saliva", the serous, mucous or mixed liquid, produced by salivary glands; and "whole saliva", the liquid collected in the oral cavity, composed by duct saliva, crevicular fluid, secretions of oral, nasal and pharynx mucous [1,4,5]. Salivary flow or flow rate (FR) is the amount of saliva produced by the salivary glands and is generally expressed in mL/min. FR can be measured via basal or Unstimulated Whole Saliva (UWS), secreted independently of the presence of stimuli, and Stimulated Whole Saliva (SWS), secreted in response to sensory stimulation[1,5].

Among the systemic conditions that may affect oral health, there are certain phases in women's life cycle, such as menopause. Menopause, as defined by the World Health Organization (WHO), is the permanent cessation of menstrual cycles due to the loss of ovarian activity. This phase is characterized by physiological changes, mostly influenced by the decrease of estrogen and progesterone, as well as by the aging of tissues[7-9]. These changes can involve numerous parts of the body, including the oral cavity[10]. This research aims at determining pH and UWS/FR in a sample of 60 menopausal women and 60 fertile women of similar mean age; detecting the epidemiological index DMFT (Decayed, missing, index of teeth) and evaluating any correlations between pH, FR age and DMFT.

Patients and Methods

Patients

After being authorized by the Intercompany Ethical Committee (N. RQ3010), patients were

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Table I. pH and FR results

		Fertile Women	Menopausal Women	Difference
pH	Average	6.86 ± 0.24	6.75 ± 0.34	0.11 ± 0.10
	Min	6.30	5.80	0.50
	Max	7.44	7.40	0.04
	D.S.R. %	3.54	4.98	1.44
	p-value	<0.05		
	DMFT correlation (R^2)	0.0083	0.0002	0.0081
FR (mL/min)	Average	0.48 ± 0.19	0.29 ± 0.17	0.19 ± 0.02
	Min	0.06	0.04	0.02
	Max	0.96	0.71	0.25
	D.S.R. %	39.62	57.39	17.77
	p-value	<0.0001		
	DMFT correlation (R^2)	0.0132	0.0047	0.0085
	pH correlation (R^2)	0.0211	0.0358	0.0147

submitted a questionnaire investigating the general health state. Exclusion criteria were: a) presence of systemic diseases that may have caused decreases in saliva production such as dry mouth or oral burning syndrome; and b) habitual use of drugs or alcohol. The mean age of the total sample (n. 120) was 50.55 ± 6.61 years (min: 40, max: 62): the mean age of the menopausal women group (n. 60) was 56.22 ± 3.69 years (min: 50, max: 62); the mean age of the fertile women group (n.60) was 44.92 ± 2.97 years (min: 40, max: 49). Patients were advised to keep a relaxed attitude and not to practice sports in the day before salivary collection. Additionally, they were asked not to eat or drink during the hour preceding the sampling procedure. UWS was collected at 11.00 a.m., via spitting method for 5 minutes in a pre-weighed, dry, deionized and sterilized tube (VACUTEST Kima® s.r.l. Arzegrande, PD, Italy), under controlled environmental temperature (mean: 23,27°C) and humidity conditions (mean: 60.08%) as measured by a barometer (PCE-THB38, PCE Group, Capannoni, LU, Italy)[1,5]. While Stimulated Whole Saliva (SWS) leads to an increase in bicarbonates and mucins – and therefore to a staggered and altered pH value – UWS is naturally produced in the oral cavity and provides a more accurate measurement. All samples and related analyses were collected over the course of 3 months (April, May and June) in order to minimize the seasonal variability of salivation. To evaluate FR we used a weighing technique (Precisa Balances Series Bj Dietikon, Switzerland). In order to avoid saliva degradation[1,5,11], pH analysis was performed using a portable pH-meter (Hanna Instruments® HI 9026, Woonsocket, RI, USA) shortly after sample collection. As descriptive statistics, we calculated: mean, minimum and maximum value, standard deviation and relative standard deviation. We used a two-tailed Student's *t*-test (*p*-value threshold set at x<0.05) to assess the existence of statistically significant differences between samples. Moreover, we calculated the correlation coefficient (R^2) among pH, FR, age and DMFT.

Our study, unlike others present in literature, was conducted following precise and standardized methods regarding unstimulated whole saliva (UWS) sampling (collection times, tools used, and time required for patient analysis and training). Sample collection was performed under controlled environmental temperature (average: 23, 27°C) and constant humidity conditions (average: 60.08%) at 11:00 a.m., since the salivary peak usually occurs around this time. While Stimulated Whole Saliva (SWS) leads to an increase in bicarbonates and mucins – and therefore to a staggered and altered pH value – UWS is naturally produced in the oral cavity and thus provides a more accurate measurement. UWS was collected via "spitting" method. Each sampling lasted for 5 minutes and took place in a pre-weighed, dry, deionized and sterile test tube (VACUTEST KIMA® s.r.l. Arzegrande, PD, Italy). All samples and related analyses were performed over the course of three months (April, May and June) in such a way as to minimize the influence of seasonal variability. Flow Rate (FR) was measured via weighing technique in order to avoid artifacts attributable to volumetric assessment. Samples were weighed by means of a technical weigh (Pre-

cisa Balances Series Bj Dietikon, Switzerland), while the pH analysis was carried out using a portable pH meter (Hanna Instruments® HI 9026, Woonsocket, RI, USA). pH sampling was carried out shortly after sample collection in order to avoid salivary degradation. Both devices were calibrated at the start of the day. Finally, all patients took part in a training designed to teach them how to avoid sample alteration. Patients were advised not to practice sports and not to perform stressful activities during the day before sample collection. They were also told not to eat or drink in the hour before. Health workers had all been trained to retrieve and handle salivary samples correctly.

Results

In the total sample, (mean age: 50.55 ± 6.61) mean pH was 6.81 ± 0.30 (min: 5.80; max: 7.44 and Directly Standardized Rate (DSR) %: 4.36) while mean FR was 0.39 ± 0.20 (min: 0.04; max: 0.96 and DSR%: 52.60). Both values were lower than what usually observed in young adults samples (mean pH: 6.95 and mean FR: 0.643)[1], which suggests an age effect on pH and FR levels. The pH and FR values of fertile and menopausal women are shown in Table I. The R^2 coefficient, resulting from the correlation between pH and age, was 0.0135 for fertile women and 0.0055 for menopausal women. Concerning FR values, our samples show a similar trend (fertile women R^2 = 0.0673; menopausal women R^2 = 0.139). Such trends are evident in Graph 1: trend lines are in continuity with each other and FR values generally tend to decrease with age. Therefore, data suggest that the decrease observed in menopausal women – being on average 11.3 years older than fertile women in our sample – may be influenced by age as pointed out by previous research[11,12]. The mean DMFT index of menopausal women was 11.93 ± 7.14 (range: 0-27) while the fertile women's one 12.23 ± 6.37 (range: 0-28). Finally, there weren't statistically significant differences and correlations between pH, FR and DMFT.

Discussion

Our results aren't comparable with most of the data previously reported due to the different protocols we used. Mean age of menopausal women (56.22 ± 3.69 years) in our study was comparable to that of most international literature studies, with some exceptions[13,14]. Fertile women had a similar mean age (44.92 ± 2.97 years), as biologically possible, to the sample of menopausal women. We chose to proceed so in order to minimize the influence of age on pH and salivary FR values as suggested by previous works[15,16]; in contrast, other studies selected way younger women for the fertile control group compared to those included in the study groups[8,13] while other did not specify the age of participants[14].

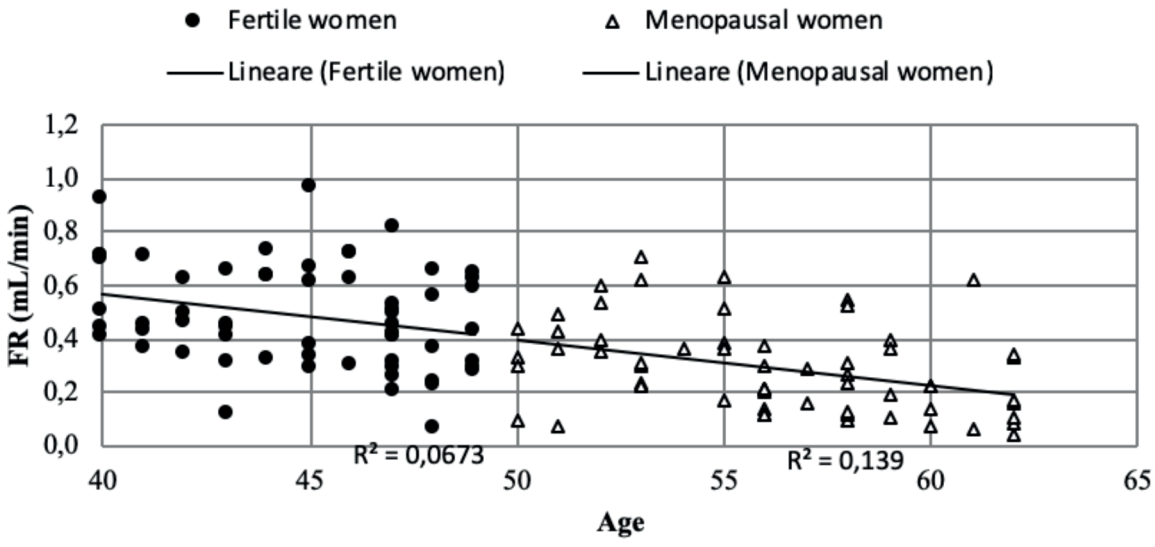

Figure 1. Comparison between the trend lines of fertile and menopausal women FR

Mean FR of our sample of menopausal women (0.29 ± 0.17 mL/min) was in line with that reported by the Agha-Hosseini et al[17,18] (respectively: 0.30 ± 0.02 mL/min and 0.35 ± 0.01 mL/min). In our study UWS/FR values of menopausal patients (0.29 ± 0.17 mL/min) was lower than those of fertile patients (0.48 ± 0.19 mL/min). Such difference (0.19 mL/min) was statistically significant (p<0.0001) and in line with most of the studies showing a similar decrease in FR that used SWS samples[8,13,15,16,19-21]. Interestingly, the study lead by Minicucci et al[14] used UWS samples and found similar FR values for the two samples. The mean age of the two groups, however, was not specified. Saluja et al[13] also didn't detect statistically significant differences (SWS) between the samples. In our research, the pH of postmenopausal women (6.75 ± 0.34) was significantly lower (0.11) (p<0.05) than that of fertile women (6.86 ± 0.24). All previous studies have also shown a decrease in pH during menopause. However, reported values of menopausal women were lower compared to those we observed in our sample: Bhat et al[8] (5.67 ± 0.49), Divya et al[15] (5.77 ± 0.37) and Saluja et al [13] (5.98 ± 0.52). Mean DMFT in our study was 11.93 ± 7.14 in menopausal women and 12.23 ± 6.37 in fertile women. Comparing these values with data from the study by Bhat et al[8] – menopausal women (8.20 ± 4.58) and fertile women (2.30 ± 2.07) – and those of the analysis of Divya et al[15] – menopausal women (5.10 ± 2.02) and fertile women (2.45 ± 0.81) – it is evident that both are lower suggesting a superior incidence of caries in our samples. Bhat et al[8] showed that DMFT was statistically superior in menopausal women (p<0,001), but no statistical difference was found in our study[8]. Finally, in our research we found no statistically significant difference in DMFT between the two groups and no correlation between a) DMFT and pH; and between b) DMFT and FR. Such result is inconsistent with what was observed by Divya et al[15] DMFT, since in their study DMFT is related to FR, but not to pH. During menopause, women go through a significant reduction in pH and FR values. These alterations, however, aren't related exclusively to physiological aging, but also to other environmental and biological factors that characterize the individual subject. Several studies have shown that when the values of UWS decrease below 0.16 mL / min patients report hyposalivation[1,2]. Such condition may cause several issues since it alters the homeostasis of the oral cavity – both at an oral and a systemic level – thus increasing the risk of developing lesions of both hard and soft tissues (stomatitis, caries, parodontopathies, etc.)[3,6]. In fact, hyposalivation causes bacterial plaque growths and an accumulation of debris that can lead to tooth decay and periodontal infections[4-7]. Salivary fluid exerts a protective buffer effect that favors the stability of pH levels, protecting the oral tissues from acids and plaque. The main salivary constituent responsible for these properties is bicarbonate, but a minor action is also carried out by phosphates and proteins[7-9]. It is shown that acid pH increases the risk of developing mycotic oral diseases. Patients with FR <0.35 ml / min have a 3.64 times greater chance of experiencing oral injury, while for those who have a pH <6.68 such chance is 2 times greater[6].

Conclusions

Prevention and early recognition of hyposalivation and reduction of oral pH are one of the tasks of health professionals. In case of FR <0.35 ml/min we recommend to a) interrupt harmful habits (smoking, alcohol drinking, etc.); b) drink a lot of water in small and frequent sips during the course of the day; c) dissolve small, sugar-free sweets to increase saliva production; d) frequently check FR and salivary pH; e) consider the use of salivary substitutes and f) undergo a microbiological tampon analysis. To patients with a pH <6.8, we recommend a) interrupting bad habits; b) adopting a diet low in acid beverages and spicy foods; c) rinsing with 5% sodium bicarbonate to increase pH levels and d) periodic FR and pH controls following appropriate protocols.

Competing interests

The authors declare that they have no competing or conflicting interests. Each author certifies that he or she has no commercial associations that might pose a conflict of interest connection with the submitted article. No funding sources supported this work.

Authors' contributions

PLFB planned the study, performed oral examinations, and wrote the manuscript. VR performed oral examinations and revised the manuscript. AN collected saliva, reviewed the literature, performed statistical analyses and wrote the manuscript. MF collected saliva and reviewed the literature.

KS collected saliva and reviewed the manuscript. AFB performed statistical analyses and reviewed the manuscript.

Consent
Written informed consent was obtained from all of the subjects.

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Ethical approval
The study was approved by our Faculty Ethics Committee n° RQ3210.

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