Comparison of two methods for sialometry: weighing and volume techniques

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ABSTRACT | The composition of saliva is essential for the oral cavity homeostasis, therefore, the decrease in salivary flow leads to consequences, such as an increase of dental caries, dry mouth and lips, dysgeusia, dysphagia, gingivitis, halitosis, mastication problems, oral mucositis, oral pharyngeal candidiasis, sleeping and speaking difficulties and traumatic oral lesions. The objective of this study was to evaluate the efficacy of the sialometry technique by weighing in comparison to the sialometry technique by volume. Fifty patients without previous complaint of xerostomia and/or hyposalivation were selected at the Oral Medicine Clinic, Dentistry School, University of São Paulo, Brazil. All samples were collected between 9 am and 10 am and the whole saliva was collect stimulated and unstimulated. Six cotton rolls were prepared, divided into three pairs and placed in different universal dispensers of a random brand, they were weighed in a previously calibrated analytical balance (FA-2104N CELTAC). The sialometry test was performed in three steps: unstimulated salivary flow, salivary flow with stimulation of 1% citric acid solution and stimulation of 1% citric acid solution every 30 seconds. The results of the weighing method were compared to the standard method. There was no significant statistical difference between the two types of collection and 100% of the participants expressed their preference for the weighing method.

DESCRIPTORS | Saliva; Salivary Elimination; Salivary Glands; Salivation.

RESUMO | **Comparação de dois métodos de sialometria: técnicas de pesagem e volume** • A composição da saliva é essencial para homeostase da cavidade oral. Assim sendo, a diminuição do fluxo salivar pode levar ao aumento da incidência de cáries, boca seca, alteração no paladar, alteração na deglutição, gengivite, halitose, problemas mastigatórios, mucosites, candidíases, problemas no sono, fala e lesões orais traumáticas. O objetivo deste estudo foi avaliar a técnica de sialometria por peso em comparação com a técnica tradicional de sialometria por volume, com coleta de saliva estimulada e não estimulada. Cinquenta pacientes com ou sem queixa prévia de xerostomia ou hipossalivação foram selecionados na clínica de Estomatologia da Faculdade de Odontologia da Universidade de São Paulo, Brasil. Todas as coletas foram realizadas entre 9 e 10 horas da manhã. Seis rolos de algodão foram separados e divididos em três pares e colocados em três diferentes coletores plásticos universais, sendo cada conjunto pesado previamente em balança analítica calibrada. O teste de sialometria foi realizado em três etapas: fluxo salivar sem estimulação; estimulado com 1% de ácido cítrico em aplicação única; e estimulado com aplicação de 1% de ácido cítrico a cada 30 segundos até completar 2 minutos. Esses resultados por peso foram comparados com o método de sialometria por volume tradicional. Não houve diferença estatística observada entre os dois métodos de coleta e 100% dos pacientes preferiram a coleta realizada com roletes de algodão.

DESCRITORES | Saliva; Eliminação Salivar; Glândulas Salivares; Salivação.

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INTRODUCTION

The salivary glands are part of the large collection of glands within the endocrine system of human beings, they are responsible for maintaining the balance of the stomatognathic system through saliva production.[1]

Saliva is essential to the homeostasis of the oral mucosa and some substances found in whole saliva help to maintain the integrity of oral tissues. Mucin is the main product of the submandibular gland, sublingual gland and minor glands, it is responsible for providing lubrication and protection for the mucous membranes.[2] Statherin is responsible for maintaining high levels of calcium available, thus improving the teeth remineralization and histatins that have antimicrobial proprieties.[3-5]

Therefore, the decrease in salivary flow can cause consequences, such as an increase of dental caries, dry mouth and lips, dysgeusia, dysphagia, gingivitis, halitosis, mastication problems, mucositis, oral-pharyngeal candidiasis, sleeping and speaking difficulties and traumatic oral lesions.[6,7]

With the increase in life expectancy, there are more complaints about the dry mouth sensation, xerostomia, from the geriatric population due to the increased use of medications, and systemic diseases.[8,9] There are studies that sought to prove that salivary flow decreases with aging, and this decrease is known to have a remarkable effect on the life quality of older adults.[9] Hyposalivation is manifested by a reduction in salivary flow and included in the etiology are Sjögren Syndrome, uncontrolled diabetes mellitus, HIV, lupus erythematosus, rheumatoid arthritis, Parkinson's disease, head and neck radiation therapy and eating disorders.[10] According to Ship et al.[11], the inhibition of acetylcholine binding to muscarinic receptors on the acinar cells is responsible for the development of an anticholinergic effect, leading to an impact on the quality and quantity of salivary output.

These malfunctions must be accurately diagnosed, and this has led to the development of easy and precise methods of diagnosis, providing fast results so an effective therapy can be implemented, and the prognosis of the malfunction can be favorable.

Sialometry is the most common method to diagnose salivary flow malfunctions. Hyposalivation, for example, is diagnosed based on the salivary flow measured by sialometry and the result must be under 0.1 mL/min of non-stimulated saliva.[1,12,13]

The objective of this study was to evaluate the efficacy of sialometry technique by weighing in comparison to the sialometry technique by volume. In addition, we also evaluated the acceptance of the method by the patient, ease of using the method and comparison of the results of both weighing and volume techniques.

METHODS

Fifty patients were selected at the Oral Medicine Clinic, Dentistry School, University of São Paulo, Brazil. This study was approved by Research Ethics Committee of the institution. All samples were collected between 9 am and 10 am, the participants were instructed not to eat, drink or brush their teeth at least 2 hours before the collection. The age of the participants ranged from 20 to 50 years. The inclusion criteria were that subjects should not present complaints of xerostomia or have used any medication that could interfere in salivary flow or of xerostomic potential such as: antihypertensive and psychotropics. All volunteers agreed to participate in this study, they signed an Informed Consent Form and answered a questionnaire regarding their habits, general health, xerostomia and medication.

Six cotton rolls were prepared, divided into three pairs and placed in different universal dispensers of a random brand. The whole set was

weighed in a previously calibrated analytical balance (FA-2104N CELTAC) with a variation of less than 0.001 g (Figure 1). Whole saliva was collected by spit method, a funnel was linked into a graduated lab cylinder (Figure 2). All samples were centrifugated to obtain the supernatant saliva that can be used for analyses.

Figure 1 | A funnel was inserted into a graduated lab cylinder to collect saliva by volume

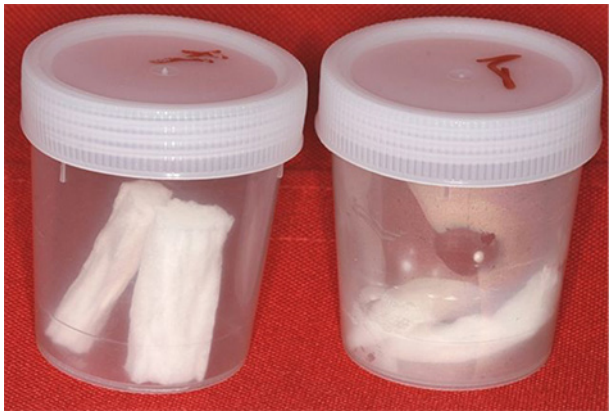

Figure 2 | Cotton rolls were prepared, divided into three pairs and placed in different universal dispensers of a random brand

The sialometry test was performed in three steps. First, the patients were instructed to swallow all saliva present in their oral cavity, then, two previously weighed cotton rolls were placed on each side of the floor of the mouth. The subject could not swallow for two minutes, the rolls were then removed and put into the universal dispenser be weighed again. Five minutes after this procedure we applied the standard method, which consisted of the patient spitting saliva into the lab cylinder for five minutes, without stimulation (P1 test). The second step was performed ten minutes later.

On the second step the salivary flow was stimulated with 1% citric acid solution. Two drops of solution were poured onto the dorsum of the tongue and the patient was asked to swallow the saliva immediately. The standard sialometry test was performed again as previously detailed (P2 test). The third step was performed ten minutes later.

The third step (P3 test) consisted of hyperstimulation of salivary production. Two drops of citric acid were poured onto the dorsum of the tongue and the patient was asked to swallow the saliva immediately. Subsequently, the last two cotton rolls were placed in the mouth of the volunteer, and for two minutes we applied two drops of citric acid in the same location, resulting in a total of eight drops. The set was weighed and the difference in weight was converted into millimeters per minute (mL/min). The standard collection method was performed with stimulation every 60 seconds for five minutes. All data obtained were converted into mL/min.

Table 1 | Differences between methods (cotton rolls × standard test)

		Cotton test	Standard Sialometry
Duration of each phase		2 minutes	5 minutes
Procedure	P1	Salivary flow without stimulation	Salivary flow without stimulation
	P2	Previous stimulation with 1% citric acid	Previous stimulation with 1% citric acid
	P3	Stimulation with 1% citric acid every 30 seconds	Stimulation with 1% citric acid every 60 seconds

RESULTS

The mean age of the volunteers was 26 years old; We analyzed 28 men and 22 women. Regarding their habits, 10 participants reported smoking, 40 reported driking socially and 10 reported no such habits. The statistical analyses were performed on paired samples, using the t-test.

There was no statistically significant difference between the two types of collection, without stimulation (p=0.84), with stimulation (2 drops before collection), (p=0.42) and with continuous stimulation (p=0.51) (Table 2). All participants (100%) expressed preference for the weighing method. Table 3 shows the general results obtained after each collection (results in mL/min).

Table 2 | Statistical test t for paired samples

Collection	Standard	With Cotton	p-value
No Stimulation	0.81 ± 0.41	0.80 ± 0.40	0.84
With Stimulation (2 drops)	1.13 ± 0.52	1.17 ± 0.53	0.42
Continuous stimulation	1.78 ± 0.69	1.83 ± 0.58	0.51

Table 3 | General results obtained after each collection (results in mL/min)

	P1 No Stimulation			P2 With Stimulation (2 drops)			P3 Continuous stimulation		
	Standard	Cotton	Dif %	Standard	Cotton	Dif %	Standard	Cotton	Dif %
Test 01	0.800	1.025	28.1	0.880	0.960	9.1	0.880	1.910	117.0
Test 02	0.320	0.430	34.4	0.280	0.680	142.9	0.280	1.690	503.6
Test 03	0.600	0.520	-13.3	0.680	1.220	79.4	1.080	2.270	110.2
Test 04	0.300	0.500	66.7	0.600	1.500	150.0	1.320	1.700	28.8
Test 05	0.600	0.250	-58.3	0.660	0.590	-10.6	1.340	1.530	14.2
Test 06	0.400	0.200	-50.0	0.500	0.300	-40.0	0.800	0.780	-2.5
Test 07	0.520	0.900	73.1	0.860	0.990	15.1	1.900	2.000	5.3
Test 08	1.000	1.000	0.0	1.200	0.990	-17.5	1.400	1.230	-12.1
Test 09	0.800	0.789	-1.4	1.000	1.043	4.3	1.600	1.925	20.3
Test 10	0.400	0.390	-2.5	0.400	0.520	30.0	1.400	1.350	-3.6
Test 11	0.600	0.905	50.8	1.000	1.132	13.2	1.400	1.980	41.4
Test 12	0.600	0.829	38.2	0.800	1.238	54.8	1.600	1.806	12.9
Test 14	1.000	0.760	-24.0	1.200	0.491	-59.1	1.800	0.927	-48.5
Test 15	1.000	0.681	-31.9	1.400	1.345	-3.9	2.000	2.617	30.9
Test 16	0.800	0.758	-5.3	1.000	1.100	10.0	1.400	1.767	26.2
Test 17	1.600	0.163	-89.8	1.800	1.147	-36.3	2.400	1.919	-20.0
Test 18	0.500	0.498	-0.4	0.640	0.597	-6.7	1.380	1.350	-2.2
Test 19	1.200	0.952	-20.7	1.200	1.518	26.5	1.600	2.182	36.4
Test 20	0.600	0.802	33.7	0.800	1.523	90.4	1.200	2.113	76.1
Test 21	0.600	0.785	30.8	0.940	1.021	8.6	1.740	1.729	-0.6
Test 22	0.700	0.885	26.4	0.840	0.911	8.5	1.540	1.494	-3.0
Test 23	0.900	0.808	-10.2	1.040	1.042	0.2	1.620	1.620	0.0

continues...

Table 3 | Continuation

	P1 No Stimulation			P2 With Stimulation (2 drops)			P3 Continuous stimulation		
	Standard	Cotton	Dif %	Standard	Cotton	Dif %	Standard	Cotton	Dif %
Test 24	1.000	0.959	-4.1	1.400	1.540	10.0	1.800	2.205	22.5
Test 25	0.800	0.770	-3.8	1.000	1.100	10.0	1.600	1.899	18.7
Test 26	0.900	1.040	15.6	0.860	0.890	3.5	1.500	1.190	-20.7
Test 27	0.149	0.152	2.4	0.395	0.353	-10.6	0.963	0.746	-22.5
Test 28	0.050	0.042	-15.4	0.153	0.128	-16.3	0.500	0.459	-8.2
Test 29	0.012	0.013	4.2	0.126	0.100	-20.6	0.412	0.399	-3.2
Test 30	0.527	0.857	62.6	0.931	1.112	19.4	1.880	1.987	5.7
Test 31	1.500	1.668	11.2	1.500	2.201	46.7	3.000	2.664	-11.2
Test 32	1.000	0.926	-7.4	1.500	1.747	16.5	2.500	2.116	-15.4
Test 33	0.500	1.320	164.0	1.000	1.726	72.6	3.000	2.309	-23.0
Test 34	1.000	0.649	-35.1	1.500	1.835	22.3	2.500	2.240	-10.4
Test 35	0.250	0.283	13.0	1.500	1.367	-8.9	1.500	2.054	37.0
Test 36	0.500	0.728	45.5	1.500	1.305	-13.0	2.500	2.339	-6.4
Test 37	1.300	1.079	-17.0	1.500	1.543	2.9	2.500	2.203	-11.9
Test 38	1.250	1.154	-7.6	2.000	1.301	-35.0	2.250	1.860	-17.3
Test 39	1.000	0.926	-7.5	1.500	1.183	-21.2	2.300	1.700	-26.1
Test 40	1.500	1.418	-5.5	1.500	1.183	-21.2	3.000	2.230	-25.7
Test 41	1.200	0.950	-20.8	1.400	0.629	-55.1	1.800	0.951	-47.1
Test 42	1.500	1.579	5.3	1.700	1.734	2.0	2.250	2.156	-4.2
Test 43	1.200	1.407	17.3	1.600	1.683	5.2	2.200	2.188	-0.6
Test 44	1.500	1.354	-9.7	2.500	2.393	-4.3	3.000	2.742	-8.6
Test 45	0.900	0.761	-15.5	2.000	2.049	2.5	2.500	2.353	-5.9
Test 46	1.300	1.241	-4.5	2.000	1.995	-0.2	2.200	2.122	-3.5
Test 47	0.900	0.900	-0.1	1.500	1.325	-11.7	2.500	2.459	-1.6
Test 48	0.459	0.489	6.5	1.200	1.028	-14.3	2.800	3.005	7.3
Test 49	1.250	1.390	11.2	1.600	1.785	11.6	2.000	2.256	12.8
Test 50	0.400	0.356	-11.0	0.600	0.564	-6.0	0.900	1.045	16.1
Mean	3.969	3.924		5.569	5.766		8.753	8.977	
Diference %	-1.1			3.5			2.5		

DISCUSSION

The participants of this study were selected by their age and health condition, the inclusion criteria were being in the same age group; having no complaints or symptoms of xerostomia or hyposalivation; or take any medications that could interfere in salivary flow; or have any basis diseases. Thus, the sample was homogenous, and the method itself could be analyzed with no external interferences. This study can be performed with different age groups, medication users, eating disorders or syndromes that affect the salivary flow.

This study was developed so every step of the technique could be observed, with no interference of the material in the final results, previous studies were affected by this. For example, a study conducted in The South Australian Dental Longitudinal Study, in which the containers were not weighed. Despite presenting differences in weigh, the saliva samples derived from different batches, which means the method must be followed precisely, so the results can be achieved, as were the results of this study.

Poll E. M et al.[13] performed a study using four commercially available saliva collection devices: Drool collected in a sterile specimen container; Salimetrics® Oral Swab (SOS); (C) Salivette® (Sarstedt) Cotton and Synthetic; Greiner Bio-One and Saliva Collection System® (GBO SCS®). The results showed significant differences on analytic level depending on the collection method. There were also significant differences on the salivary flow rates depending on the saliva collection method. This study used the same conditions and inclusion criteria as our study, however the focus was on saliva collection devices to quantify proteins present in saliva and to provide levels for C-reactive protein (CRP), myoglobin, and immunoglobulin E (IgE) on the saliva of healthy individuals.

Michishige et al.[14] compared three methods (suction method, spitting method and swab method) to collect saliva, in this study the samples were collected from 2 pm to 3 pm, however the interval of collection was the same in both studies. The results were different, since the Michishige study was influenced by the circadian rhythm, which did not happen in our research.

There was a discussion regarding the accuracy of the method, as the collection by volume presented incoherencies due to the bubbles formed in the tube of saliva, interfering in the final measurement, however, the results showed no differences. The method proved to be easy on patients with masticatory difficulties resulting from aging or tooth loss. We used 1% citric acid instead of pre-softened polyvinyl acetate gum or paraffin wax, thus, there were no difficulties or discomforts caused by chewing.

Previous studies[1-7,8] showed that the differences in the final results were due to the different methods, age, exclusion and inclusion criteria, however this study demonstrated that the method did not interfere in the final results when collecting saliva to measure salivary level. Nevertheless, other factors, such as the design of the studies may have an influence, reinforcing the idea that what influences the results is not the method, but the sample studied.

CONCLUSION

The conclusion of this study is that the method did not influence the final measurement results.

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