STANDARDIZATION OF A SIMPLE METHOD TO STUDY WHOLE SALIVA: CLINICAL USE IN DIFFERENT PATHOLOGIES

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ABSTRACT

The present study describes a methodology to assess the salivary flow rate in humans. Whole saliva was obtained from the floor of the mouth with a plastic dental ejector and a vacuum pump.

Forty healthy subjects of both sexes and 51 patients with different pathologies (Sjögren Syndrome, Thyroid Dysfunction, Diabetes Mellitus) were included in the study.

It was demonstrated that basal salivary flow rate was stable five minutes after the insertion of the oral ejector. Salivary flow rate did not show significant differences between sexes and was independent of the negative pressure level of the vacuum pump.

Stimulated salivary flow rate was quantified over a period of 3 minutes, starting 5 minutes after the introduction of the oral device. The stimulus was paper filter disks soaked in citric acid (2%) placed on the tongue dorsum.

The use of this method confirmed the reduction of salivary flow rate in patients with Sjögren Syndrome. In addition, a significant reduction in salivary flow rate was observed in patients with primary thyroid insufficiency and peripheral neuropathy secondary to Diabetes Mellitus.

***Key Words**: Whole saliva, Sialometry, Sialochemistry, Sjögren Syndrome, Hypothyroidism, Diabetes Mellitus.*

ESTANDARDIZACIÓN DE UN MÉTODO SIMPLE DE RECOLECCIÓN DE SALIVA TOTAL. SU APLICACIÓN CLÍNICA

RESUMEN

En este trabajo se describe la normatización de un método para determinar flujo salival en humanos utilizando saliva total obtenida del piso de la boca mediante un eyector dental descartable y una bomba de vacío (equipo dental).

En este estudio se evaluaron 40 sujetos sanos de ambos sexos y 51 pacientes con diversas patologías (Síndrome de Sjögren, Disfunción Tiroidea, Diabetes Mellitus).

Se demostró que el flujo salival basal era estable a partir de los primeros 5 minutos de colocado el eyector en la cavidad bucal. No se encontraron diferencias significativas en el flujo salival basal comparando los sexos, siendo independiente de la intensidad del vacío efectuado por la bomba. El flujo de saliva total estimulada fue determinado durante 3 minutos, luego de los primeros 5 minutos de colocado el eyector en la boca. El estímulo se efectuó adosando en la cara dorsal de la lengua discos de papel absorbente, embebidos en ácido cítrico al 2 %.

El uso de este método en pacientes con Síndrome de Sjögren confirmó la reducción del flujo salival respecto a los sujetos sanos. Los pacientes hipotiroideos y con neuropatía diabética demostraron disminución del flujo salival.

***Palabras Clave:** Saliva Total, Sialometría, Sialoquímica, Síndrome de Sjögren, Hipotiroidismo, Diabetes Mellitus.*

INTRODUCTION

The clinical importance of chemical determinations in saliva is increasing. They have been used for assessing endocrine functions (1), for monitoring drug concentrations (2), for measuring antibodies and antigens (3), and for dynamic diagnostic tests (4).

Saliva may be obtained separately from each pair of glands such as parotid (5, 6), submaxillary / sublingual (7, 8), palatine (9), and smaller glands (10), but these techniques should be performed by specialized technicians. Besides, because the participation of each group of glands in the composition of whole saliva is very variable, the study of whole saliva is now preferred (11).

Four collection methods have been devised to measure resting whole saliva: draining, spitting, suction and swab (with cotton rolls or filter paper). They

are considered to be equivalent (11). Gustatory or masticatory stimuli are used to stimulate the salivary flow (11). The aim of this paper was to describe a simple, accurate and reliable method to collect resting and stimulated saliva in order to detect changes in whole saliva composition secondary to primary systemic disease.

MATERIALS AND METHODS

Whole saliva was obtained from the floor of the mouth using a plastic dental saliva ejector tip (a), attached via plastic tubing (b) to a vacuum pump (c). Saliva was collected in a graduated centrifuge tube (d) as shown in Figure 1. This methodology, based on Navazesh and Christman (11), was designed for easy application in hospitalized and ambulatory patients.

Saliva was collected from 40 healthy subjects (10 males and 10 females; 35 ± 15 years old). They were non-smokers and were off alcohol and drugs.

Resting whole saliva was obtained in basal conditions between 8:00 and 9:00 a.m., in order to minimize changes due to circadian variations. Before the extraction of saliva the subjects rinsed their mouth twice with tap water to remove food debris and other non salivary elements that could interfere with measurements.

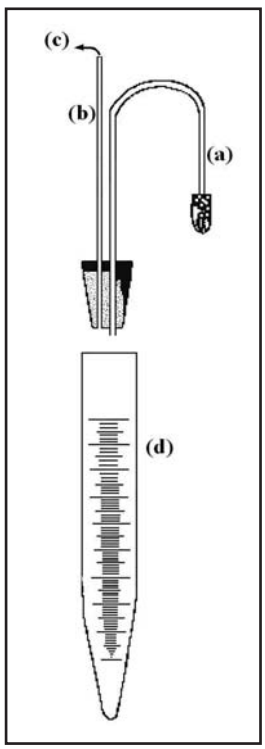

Fig 1: *Saliva collection device: its components.*
a) plastic dental saliva ejector tip;
b) plastic connecting tube;
c) vacuum pump;
d) graduated centrifuge tube.

Stimulated whole saliva was measured the next day. It was collected during a 3-minute period, 5 minutes after the insertion of the ejector tip in the mouth. The stimulus consisted of 3 Whatman filter paper disks, 18 mm in diameter, soaked in citric acid (2%), placed on the tongue dorsum for one minute each.

Study design

a) Optimal time interval for saliva collection

Resting whole saliva was obtained from the 40 control subjects, over a 15-minute period, at one-minute intervals.

b) Influence of different negative pressures of the vacuum pump on saliva collection

Six control subjects were selected, 3 with high saliva resting flow rates and 3 with low saliva resting flow rates. The extraction of saliva was performed at different negative pressures of the vacuum pump (8, 10, 14, 17, 20 and 23 mm Hg).

c) Reproducibility of the results

Resting whole saliva samples were obtained daily for 10 days from 10 control subjects.

d) Storage conditions and sialochemistry

The samples obtained from healthy subjects were centrifuged and the supernatant was divided in two parts. One was immediately frozen and kept for 30 days at -22 ºC until assays were performed. The other was kept at room temperature and processed within 30 minutes. Total proteins, amylase activity, chloride, calcium, sodium and potassium were assessed in all salivary samples as described (12, 13, 14, 15).

e) Clinical application

The study group consisted of 51 patients, 13 with thyroid disease, 25 with Diabetes
Mellitus and 13 with Sjögren Syndrome.

Of the thirteen female patients with thyroid disease (aged 45 ± 20 years), six patients had primary hypothyroidism (HT-) and seven had hyperthyroidism (HT+).

Of the twenty-five patients with Diabetes Mellitus (9 women and 16 men; aged 25 ± 8 years), eighteen had Diabetes Mellitus without neuropathy (DM N-), and seven had Diabetes Mellitus with neuropathy (DM N+).

The thirteen patients with Sjögren Syndrome (SS1) (aged 50 ± 15 years), were diagnosed by biopsy grade 4 as described by Greenspan et al. (16). Nine had associated rheumatoid arthritis (SS2).

Statistical methods

The results were expressed as mean ± SEM. The data were analyzed by ANOVA. When the hypothesis of the equality of the means was rejected, Student-Newman–Keuls test was employed (17). A value of $p < 0.05$ was considered significant.

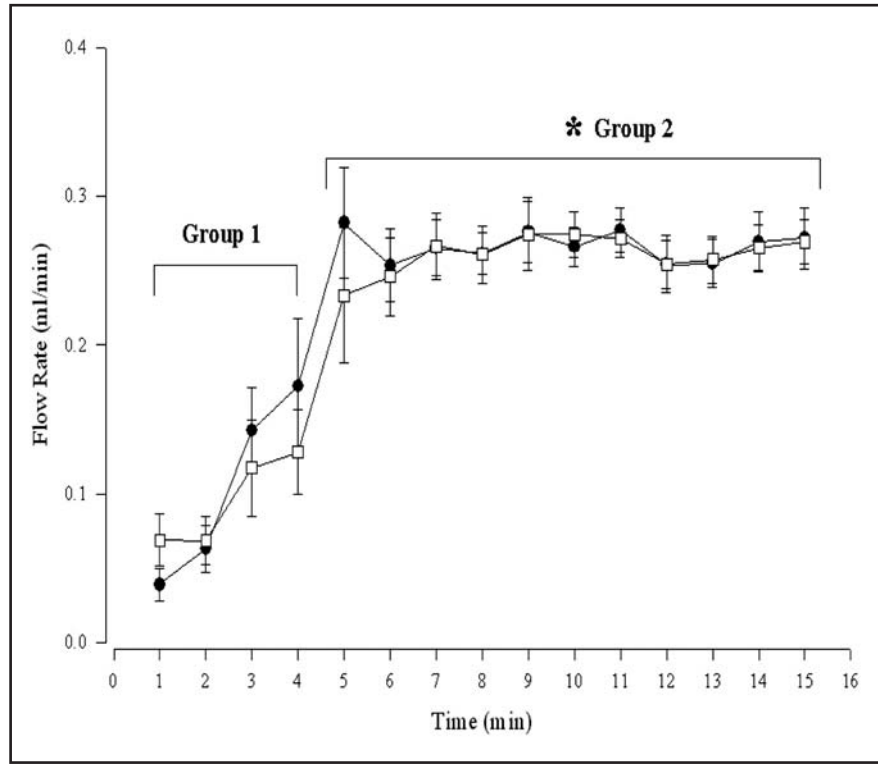

Fig 2: *Basal whole salivary flow rate in 40 healthy subjects obtained over a fifteen minute period (20 males ●-● and 20 females □-□).*
Salivary flow-rate obtained from minutes 1 to 4 (group 1) was unstable and significantly lower ($p < 0.0001$) than after 5 minutes (group 2).

RESULTS

The resting whole saliva flow rates obtained every minute for 15 minutes in 40 healthy subjects are shown in Figure 2. Resting salivary flow rates were significantly different ($p < 0.05$) between Group 1 (1 and 4 minutes of collection) and Group 2 (from 5 to 15 minutes of collection). These differences were unrelated to gender.

Whole saliva flow rates stimulated by citric acid did not show differences between males (1.56 ± 0.33 ml/min) and females (1.57 ± 0.58 ml/min).

Table I shows the saliva volumes obtained over a 5 minute period (5 to 10 minutes after saliva collection began). Volumes were independent of variation in negative pressure of the vacuum pump and of variations in basal flow rate high (>0.28 ml/min) or low (< 0.28 ml/min).

Table II shows that whole saliva resting flow rate did not change in healthy subjects over a 10-day period.

Table III shows salivary levels of total proteins, amylase activity, sodium, potassium, chloride and calcium. No significant differences were found as a result of freezing the samples.

Resting and stimulated whole saliva flow rates of patients are displayed in Table IV. No difference was found between DM N patients and controls. DM N+ patients showed a significant reduction in the stimulated flow rates ($p < 0.001$).

TABLE I. Effects of vacuum pump pressure on whole saliva resting flow rate in healthy subjects with high salivary flow rate (> 0.28 ml/min) or low salivary flow rate (< 0.28 ml/min)

VACUUM PUMP PRESURE (mm Hg)	HIGH FLOW RATE (ml/min)	LOW FLOW RATE (ml/min)
8	0.305 ± 0.003	0.253 ± 0.015
10	0.306 + 0.003	0.254 ± 0.006
14	0.306 ± 0.004	0.255 ± 0.007
17	0.311 ± 0.005	0.263 ± 0.014
20	0.295 ± 0.008	0.253 ± 0.018
23	0.307 ± 0.001	0.269 ± 0.004

Salivary flow rates were independent of the vacuum pump pressure

TABLE II. Whole saliva flow rates of 10 subjects measured during 10 consecutive days

SUBJECT	FLOW RATE (ml/min)
A	0.26 ± 0.01
B	0.27 ± 0.01
C	0.27 ± 0.01
D	0.26 ± 0.01
E	0.27 ± 0.01
F	0.28 ± 0.01
G	0.23 ± 0.01
H	0.27 ± 0.01
I	0.27 ± 0.01
J	0.29 ± 0.01

Salivary flow rates are expressed as mean ± SE.
Variability of salivary flow-rates in healthy subjects was not significant.

TABLE III. Storage conditions and sialochemistry.

	Unfrozen samples	Frozen samples
Protein (mg %)	228 ± 21.30	210 ± 19.90
Amylase (UA)	252 ± 27.60	335 ± 21.90
Na^{++} (mEq/l)	3.75 ± 0.79	3.80 ± 0.80
Ca^{++} (mEq/l)	2.22 ± 0.21	2.35 ± 0.40
K^{+} (mEq/l)	21.3 ± 0.55	22.3 ± 0.40
Cl^{-} (mEq/l)	19.8 ± 2.80	20.0 ± 1.00

After centrifugation the supernatant of saliva samples was divided in two parts: one part was processed within 30 minutes (unfrozen) and the other was stored at -22 ºC during 30 days until processing (frozen).
Levels of protein, amylase activity and electrolytes were measured in supernatants of saliva samples. All values were expressed as mean ± SE.
No significant differences were found between frozen and unfrozen samples.

Patients with hypothyroidism exhibited a significant reduction in resting ($p < 0.05$) and stimulated ($p < 0.01$) flow rates compared to healthy subjects. No differences were found between patients with hyperthyroidism and controls.
A decrease in resting ($p < 0.05$) and stimulated salivary flow rate ($p < 0.001$) was confirmed in patients with Sjögren Syndrome. No difference was found between SS1 and SS2 patients.

DISCUSSION

This study shows a simple and practical method to measure whole saliva flow-rate in humans. The equipment is easily obtainable, as it can be found in dental offices and clinical laboratories. The samples should be collected five minutes after the introduction of the oral device to obtain stable salivary flow rates. At this time, values were accurate and independent of the intensity of suction of the vacuum pump.
This method is useful for sialochemistry assays. Saliva samples should be centrifuged and the supernatant used to determine electrolytes, amylase activity and proteins. Freezing of the samples for 30 days did not alter the results.
Salivary flow rate evaluates acinar gland function and is useful for the study of functional gland integrity in different clinical disorders. It is known that salivary flow rates are decreased in Sjögren Syndrome (18, 19, 20, 21) and using the methodology proposed we reconfirmed this finding Diabetic patients did not show changes in whole saliva resting or stimulated flow rates. However, this finding is still controversial (22, 23, 24, 25). The decrease in whole saliva flow-rates in diabetic patients is suggestive of neuropathy and it has been proposed that this finding contributes to the diagnostic suspicion of this neurological complication (26, 27). The saliva flow rates are decreased in hypothyroid, but not in hyperthyroid patients. These findings are in keeping with studies on experimental models that revealed that thyroid hormones modulate the effects

TABLE IV. Basal and stimulated whole saliva flow rate in patients with several pathologies.

FLOW RATE	SJOGREN PATIENTS		THYROID PATIENTS		DIABETIC PATIENTS	
(ml/min)	SS1	SS2	HT(+)	HT(-)	DM N-	DM N+
Basal	0.07 ± 0.04*	0.10 ± 0.04*	0.30 ± 0.03	0.11 ± 0.04*	0.23 ± 0.03	0.17 ± 0.04
Stimulated	0.38 ± 0.15*	0.43 ± 0.16*	1.75 ± 0.31	0.78 ± 0.28*	1.47 ± 0.12	0.97 ± 0.23*

Flow-rate (ml/min) is expressed as mean ± SEM
SS1: patients with Sjögren Syndrome; SS2: patients with Sjögren Syndrome and associated Rheumatoid Arthritis.
Patients with primary Hypothyroidism (HT-): and Hyperthyroidism (HT+):
Patients with Diabetes Mellitus without neuropathy (DM N-) and with neuropathy (DM N+).
Healthy subjects: basal flow rate: 0.27 ± 0.02 ml/min; stimulated flow rate: 1.54 ± 0.12 ml/min
* P< 0.05 patients compared with healthy subjects

of autonomic drugs and neurotransmitters on salivary glands (28, 29, 30).

This study reveals that the method proposed is a useful tool that contributes to the diagnostic approach to systemic diseases. Further studies should be considered to extend the diagnostic importance of this non-invasive methodology to other areas of internal medicine.

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