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Obtaining Parotid Saliva Specimens After Major Surgery

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Abstract

The purpose of this study was to develop and test a standard method of collecting saliva from postoperative patients. Saliva was collected from patients following major abdominal surgery from both parotid glands, in intraoral cups and measured in milliliters. Collection time was measured with a stopwatch and flow rate was calculated by dividing the amount in milliliters by the minutes. Trained research nurses stimulated saliva production with lemon juice and collected saliva at four time points on postoperative day 2. Attrition was 9% due to ineligibility after enrollment and one withdrawal. In participating patients ($n = 68$), there were 272 tests planned and 28% were missing. The reasons were postoperative health problems, hospital discharge, and not wanting to be bothered. When saliva collection attempts were made, three-fourths were successful, but the remainder resulted in “dry mouth.” Milliliters, minutes, and flow rate were calculated with and without those with dry mouth. Mean flow rates were .23 to .33 ml/min excluding those with dry mouth, and .17 to .24 ml/min including those with dry mouth. Saliva variables were correlated with antihypertension medications, opioids, opioid side effects, and length of surgery, but correlations were not found consistently at all four time points. The findings suggest that nurse researchers studying biological markers can successfully collect saliva from postoperative patients if they recognize the difficulties and make efforts to minimize and control for them.

Keywords

saliva; collection flow rate; parotid; stimulated postoperative

*Corresponding author; origin of study.

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Saliva is considered a relatively accessible biologic fluid that can be obtained by noninvasive means and analyzed for biological markers of health. This information is important to researchers who wish to study salivary factors in postoperative patients. The two parotid glands account for 25% of the daily production of saliva while the submaxillary glands contribute 70% (Wotman & Mandel, 1976). The normal salivary flow rate of each parotid gland is less than .05 ml/min without stimulation but 10 times as fast (.5 to .7 ml/min) when stimulated (Wotman & Mandel, 1973; Wotman & Mandel, 1976). Eating is an important stimulus of saliva flow. Based on findings, Watanabe and Dawes calculated that 570 ml of whole saliva is produced during a 24-hour day with 39% produced during an hour of eating, 54% at other times during the day, and 7% throughout the night (1988). Additionally, parasympathetic stimulation results in saliva production and sympathetic stimulation results in restricted blood supply to the gland, and this inhibits saliva production (Turner, 1993).

Most studies of saliva collection have been done only with healthy persons (Miletic, Schiffman, Miletic, & Sattely-Miller, 1996; Watanabe, Iglehart, & Bolognesi, 1983). Accurate information of the flow rate of saliva following major surgery would be useful to both researchers and also to clinicians. Patients who undergo major surgery are physically and emotionally stressed by a cascade of events: anxious anticipation, anesthesia, surgical trauma, hospitalization, and pain (Bergman et al., 2001). Effects of nursing interventions to reduce postoperative stress and pain can be evaluated less painfully with salivary indicators as compared to those from plasma. However, situational factors complicate data collection. For example, patients are not permitted to eat or drink in the early postoperative period. This greatly reduces saliva production.

Nevertheless, in five studies, investigators have successfully collected whole saliva before and after major surgery. Two of these measured saliva in patients before and after hysterectomy. Lahteenmaki, Salo, & Tenovu (1998) stimulated whole saliva flow by asking participants ($N = 30$) to chew paraffin. They collected the saliva by asking participants to drool into a container. Saliva flow decreased 73% from 1.1 ml/min before surgery to .3 ml/min on the first day. Smith-Hanrahan (1997) collected unstimulated whole saliva with a Q-tip under the tongue ($n = 16$) and weighed the Q-tip before and after collection. Saliva decreased 93% from $1.6 \pm .5$ ml/min preoperatively to .1 ml/min on postoperative day 1. Measuring saliva daily, she found that from day 1 to day 3, the flow rate remained lower than preoperative values. Both of these hysterectomy studies found that the flow rate returned to nearly presurgical levels by day 4.

Saliva was also measured in two investigations with open-heart surgery patients; no anticholinergic drugs were prescribed in either study. In one study ($n = 18$), by Lahteenmaki, Tenuvuo, Salo, & Pertila (1997), the paraffin-stimulated flow rate of whole saliva decreased after surgery by 70%, from 1.8 ml/min to .4 ml/min on the second and third postoperative days and returned to pre-surgery levels on the 7th day. In the other study, Bergmann and colleagues (2001) were able to collect adequate samples of whole saliva from 60 patients before surgery and again on day 1 using a cotton dental roll (Salivette)[®], but they did not report the amount of saliva or the flow rate.

Saliva flow decreased after minor surgery as well. In 20 persons undergoing minor orthopedic surgery for knee ligament injuries, saliva was collected using Salivettes[®] at 8:00 a.m. on the day of surgery and at the same time on the first postoperative day. Pre to postoperative saliva secretion decreased significantly from .26 ml/min on the day of surgery to .18 ml/min the day after ($p < .001$) (Karkela, Vakkuri, Kaukinen, Huang, & Pasanen, 2002).

Investigators in all of these studies collected whole saliva. However, when obtaining whole saliva, the flow rate and constituent values are unreliable because of the variable amounts contributed by the parotid, submandibular, sublingual, and minor glands. In addition, the

chemistry of food particles, bacteria, shed cells, and leukocytes found in the mouth may affect results. Measurement of immunoglobulins produced in the saliva glands may be confounded by those in the plasma that can seep into the mouth in gingival crevicular fluid. This fluid is found in periodontal disease and after injury, even from vigorous tooth brushing. Collection of parotid saliva avoids these problems (Jackson, Mestecky, Moldoveanu, & Spearman, 1999; Tabak, 2001; Wotman & Mandel, 1976). This paper reports the amount, collection time, and flow rate of parotid saliva collected from subjects in a large study of postoperative abdominal surgery patients (Good, Anderson, Albert, & Wotman, 2001–2005).

Materials and Methods

Measures

Saliva was collected from both parotid glands simultaneously in intraoral (I/O) cups as described by Jackson, et al. (1999). The I/O cups were obtained from Annie Hooks (312 22nd St. SW, Birmingham, Alabama 35211; phone 205-923-2983). Saliva was collected twice between 10 and 11 a.m. and again between 2 and 3 p.m. on postoperative day 2. A stopwatch was used to measure the time in minutes and seconds that the cups were in the mouth. The saliva was placed in a graduated collection tube and measured in milliliters. Flow rate was calculated by dividing the milliliters by time in the mouth (Good et al., 2001–2005). Patients were interviewed for demographic and surgical variables and rated the intensity of pain sensation and distress on visual analogue scales and they rated intensity of opioid side effects on an opioid side effects scale (Good et al., 2001–2005).

Patients were classified in two groups according to whether or not they had opioids in effect at the time of the test. Patients with opioids in effect included those who had used their patient controlled (PCA) pump during the hour before the test, those used it during the test, and those who had received oral or intramuscular opioids prior to the test. Pump values were obtained before and after each test and other opioids (usually oral opioid/nonopioid combinations), anticholinergic medications and length of surgery were obtained from the hospital record.

Research procedure

Approval was obtained from the Institutional Review Board of the university and the hospital and each subject provided written informed consent preoperatively in the surgical holding area. Research nurses were trained to collect the saliva. Initial explanations were followed by instruction and practice with supervision by Stephen Wotman, DDS in the dental clinic. The nurses practiced the procedure repeatedly on colleagues until they mastered the technique. Each demonstrated accurate placement in the oral cavities of different people. In the clinical setting, they first observed and then obtained saliva under supervision until they demonstrated competence and expressed confidence in doing the procedure.

After surgery, saliva was collected four times using a standard procedure. Subjects were asked to rinse their mouth three times with tap water and sit in an upright position. A flashlight was used to visualize Stenson's duct on the inner wall of the cheek opposite the upper third molar. Using the correct size for the person's oral cavity, an intraoral cup was placed with the rounded side next to the teeth and the opening in the flat side over the orifice of Stenson's duct. The straight bottom of the opening was kept parallel to the floor of the mouth. Gentle pressure was applied to the external surface of the cheek to cause a small amount of air to be expressed from the cup, creating a slight negative pressure that helped to keep the cup in place. This procedure was then repeated on the other cheek. A drawing of the intraoral cup can be found in Schaefer et al. (1977).

A pilot study indicated that there was insufficient saliva on the first postoperative day. Therefore, to increase the success rate, saliva sampling was done on the morning and afternoon of day 2. Since lemon juice stimulation improved saliva collection in the pilot, saliva flow was stimulated with a small amount of lemon juice from an individual packet available in grocery stores; this was applied with a cotton applicator to both sides of the tongue three times: just after the intraoral cups were placed and 1 and 2 minutes after that. Investigators have reported that stimulated saliva is preferable to unstimulated because it can be collected more easily and is less adversely affected by storage (Brandtzaeg, 1989), although Jackson et al. (1999) reported that it results in diluted saliva and variation in flow rate.

A stopwatch was used to time the flow rate in minutes and seconds, and universal precautions were used. The cups were carefully removed when they were full. When clear of the lips, the cup was laid on a paper towel on the over-bed table with the round side down and the opening face up, so the contents would not spill. The data collector transferred saliva with a pipette to a graduated tube and recorded the amount. The tubes were immediately placed on ice and taken to the lab. The saliva was frozen at -70°C .

Missed tests occurred for reasons that could be expected in postoperative patients (Table 1). Of the 68 subjects on day 2 in the morning, 12 (18%) missed the first test and 18 (27%) missed the second test; in the afternoon, 21 (31%) missed the first test and 27 (40%) missed the second test (Table 2). The two most frequent reasons were that patients did not want to be bothered and there was conflict with preparations for hospital discharge.

Results

Saliva was collected from the first 75 postoperative abdominal surgery patients in the larger study. Six patients were disqualified from data collection because of ICU transfer, epidural anesthesia, or cancelled surgery, and one withdrew from the study.

In the final sample of 68 subjects, the mean age ($\pm$ SD) was 47 ± 10 . The majority of subjects were female (82%), white (59%), married (59%), employed (77%), non smokers (84%), non drinkers (94%), with no diagnosis of cancer (93%), with at least 1 year of college (71%) and with an income of $< \$50,000$ (61%). Two-thirds ($n = 41$, 60%) underwent gastrointestinal surgery; one-third ($n = 26$, 38%) underwent gynecological surgery, and 1 had urologic surgery.

The success rate for collecting at least .1 ml of parotid saliva on day 2 in the morning was 77% at the first test and 76% at the second test. The success rate in the afternoon was 74% at first test and at 78% at the second test. In addition to success rates, Table 2 gives the mean amount of saliva, the minutes of collection time, and the flow rate of parotid saliva on postoperative day 2 in the morning between 10 and 11 a.m. and in the afternoon between 2 and 3 p.m. Values in the upper part of the table represent only subjects who were able to provide $\geq .1$ ml of saliva, excluding those in whom collection was attempted but who had a "dry mouth." Values in the lower part of the table are for all subjects, including those in which collection was attempted but no saliva was obtained (dry mouth included). Only values in the upper part of the table will be used for future analysis of components of saliva, because flow rate can be calculated only in those who produced saliva (Table 2). Values in the lower part of the table were used in this study to examine correlations with variables such as medications that are thought to affect flow rate.

The upper part of Table 2 indicates that in patients who provided saliva, the mean milliliters of saliva at each data point ranged from $.90 \pm .87$ to $1.10 \pm .85$ ml. The mean time needed to collect the saliva ranged from 3 minutes, 54 seconds $\pm 1:20$ to 4 minutes and 27 seconds $\pm 1:07$. The mean flow rates ranged from $.23 \text{ ml/min} \pm .23$ to $.33 \text{ ml/min} \pm .26$. Thus, means were

fairly stable, but standard deviations were relatively large across the four tests (Table 2, Figure 1).

The lower half of Table 2 indicates that means of milliliters, minutes and flow rate were smaller when patients with dry mouth were included. Gender was not correlated with flow rate, perhaps because the number of males was low (16%); however, males had consistently greater mean flow rates than females in all of the four saliva tests (.03 to .20 ml/min more). Significant correlates of saliva at several points were anti-hypertension medications, $r = -.27$ to $-.41$; pain, $r = -.33$ to $-.52$; opioids in effect at the time of the test, $r = -.27$ to $-.40$; opioid side effects, $r = -.29$ to $-.38$; and length of surgery, $r = -.31$ to $-.33$; $p < .05$ to $< .01$. The negative correlations indicate that more anti-hypertension medications, more pain, more opioids in effect at testing, more opioid side effects and longer surgery were related to lesser amounts of saliva in milliliters, collection time and/or flow rate.

Discussion

We found that when we used a standard method of stimulation and collected saliva on day 2 in the morning and afternoon, three-fourths of our postoperative patients were able to produce enough saliva for testing ($\geq .1$ ml). This provides some support for the feasibility of collecting saliva in postoperative populations. Although there were large standard deviations in milliliters and flow rate, mean milliliters were stable across data points. We found an increase in flow rate in the afternoon and a decrease in collection time (Table 2). These findings point to the reliability of the method and its consistency with the diurnal rhythms of saliva flow.

In these patients recovering from major abdominal surgery, missed tests occurred because of nausea, sleeping for long periods, being away from the unit, and leaking intravenous tubing (requiring time to repair). Most patients who missed a test due to hospital discharge had undergone one type of surgery--gastric bypass and were discharged earlier than we had expected.

Since only 25% of total saliva is from the parotid gland (Wotman & Mandel, 1976), we expected that the parotid flow rate after surgery would be less than the whole saliva flow rates reported in previous studies. And indeed, our rate was lower than that for whole saliva in hysterectomy and cardiac patients (Lahteenmaki et al., 1998; Lahteenmaki et al., 1997; Smith-Hanrahan, 1997), but it was greater than that for whole saliva collected following minor knee surgery (Karkela, et al., 2002) (Table 3).

To maximize the amount of saliva and minimize the collection burden for postoperative patients, we obtained saliva simultaneously from both parotid glands of postoperative patients; thus the flow rates reported here are pooled rates. Other investigators have reported flow rates from individual parotid glands in healthy persons (Table 3). Mandel and Khurana (1969), for example, used a modified Curby cup (Curby, 1952) and found a graded parotid flow rate as they increased the concentration of citric acid stimulation from .5% to 1% to 2% in healthy adults. Fischer and Ship (1999) stimulated saliva production with 2% citric acid and using a modified Carlson-Crittenden cup, collected parotid saliva from younger and older healthy unmedicated adults over three hourly points in the morning. Our study was designed to standardize a valid saliva collection method that would obtain sufficient saliva for analysis.

Using cotton swabs, passive drool, or sublingual aspiration, whole saliva might have been collected more comfortably for our postoperative patients and more conveniently for our data collectors (Navazesh, 1993). They could also have collected a greater volume of whole saliva in a shorter time, because of the contributions from sublingual and submandibular glands. However, whole saliva can contain plasma-derived proteins due to periodontal inflammation and leakage, and these would confound the results of constituent analyses (Jackson et al,

1999). Collection of submandibular saliva involves fashioning individual collectors (Block & Brotman, 1962), while parotid cups are ready-made. Sampling only parotid saliva controls for differences in constituents among the saliva glands and prevents contaminants from food, cells, and gingival crevicular fluid (Personal Communication, Michael E. Lamm, MD, Stephen Wotman, DDS, June, 2002; (Brandtzaeg, 1998; Jackson et al., 1999).

Knowledge of saliva collection from postoperative patients is important because saliva can be used to study drugs, hormones, antibodies, DNA, oral and systemic disease and its progression, and patient compliance and lifestyle choice (Tabak, 2001). It is important for nurse researchers consider the relative ease of saliva collection from healthy persons compared to postoperative individuals and the possibility of blood as an alternative source of biological markers. Painless blood collection is feasible through indwelling intravenous access devices and is important for pain studies. In our postoperative patients, however, many of these devices were discontinued on the first day. Compared our surgical studies, saliva could be collected more easily from outpatients and there would be greater patient tolerance.

The rates of success, milliliters, minutes, and flow rates found in our study provide concrete information for researchers to use when planning sample size, attrition rates, and saliva collection methods. The type and amount of stimulation should be the same for all patients. In addition, researchers may wish to exclude patients undergoing long surgeries and those taking antihypertension medication or other drugs that affect saliva flow (Wotman & Mandel, 1976). However, they must consider the effect of such exclusions on accrual goals. Prognostic factors can also be balanced across the groups with computerized minimization (Zeller, Good, Anderson, & Zeller, 1997) or recorded and controlled statistically.

Findings are limited by the sample size, the number of men, and the correlates that were studied. Additional research is needed to confirm these findings with larger samples of postoperative patients. Results can be extended by measuring a broader range of variables that could affect saliva flow, such as antidepressants, antihistamines, anticholinergics, and antiemetics, and also measuring conditions that affect saliva flow such as diabetes, hypertension, depression, and post-menopause (Pankhurst et al., 1996; Wotman & Mandel, 1973; 1976).

Because one fourth of our patients produced no saliva and the rest had a diminished flow rate, it is recommended that postoperative nurses consider the potential for infection and abrasion (Fischer & Ship, 1999; Wotman & Mandel, 1976). The findings underscore the importance of providing gentle and thorough oral care to relieve discomfort and protect dry oral mucosal surfaces until oral intake is resumed. Manipulation of oral tissues should be done with care when using tongue blades and swabs and while intubating and suctioning patients.

This study demonstrated that saliva collection in postoperative patients is feasible if difficulties can be managed. The authors recommend collecting parotid saliva and measuring the milliliters and time to calculate the flow rate to use as a covariate when analyzing constituents.

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Figure 1.

The flow rates with and without the subjects with dry mouth are parallel. The flow rate is lower when those with dry mouth were included. There was some increase in flow rate from morning to afternoon.

Table 1

Main Study: Reasons for Missed Tests Day 2 (N = 68)

Reason	Day 2 AM		Day 2 PM	
	1 st test	2 nd test	1 st test	2 nd test
Too ill	0	0	1	2
Intravenous infusion leaking	0	0	1	1
Away from the unit	2	2	1	1
Slept for long periods	2	2	2	2
Nausea	5	5	1	1
Did not want to be bothered	3	6	5	9
Hospital discharge	0	2	10	11
Total	12 (18%)	18 (27%)	21 (31%)	27 (40%)

Note: The total number of missed tests increased by the afternoon the number of subjects with missing data because of hospital discharge or not wanting to be bothered increased by day 2, while the numbers of those with missing data because of illness, nausea or being away from the unit decreased.

Table 2
Day 2 Stimulated Saliva: Success, Milliliters, Minutes, and Flow Rate N = 68

		Success		Milliliters			Minutes (mm:ss)			Flow rate (ml/min)		
Test	Attempts	<u>n</u>	<u>%</u>	<u>M</u>	<u>SD</u>	<u>Range</u>	<u>M</u>	<u>SD</u>	<u>Range</u>	<u>M</u>	<u>SD</u>	<u>Range</u>
Subjects with dry mouth excluded ^a												
AM												
1 st test	56	43	77%	1.10	.85	.1–3.5	4:20	1:00	2:10–6:30	.28	.24	.02–.86
2 nd test	50	38	76%	.90	.87	.1–4.4	4:27	1:07	2:11–8:00	.23	.23	.02–.88
PM												
1 st test	47	35	74%	1.04	.63	.2–2.5	3:54	1:20	2:00–6:45	.33	.26	.03–.89
2 nd test	41	32	78%	1.03	.86	.1–3.0	4:03	1:28	1:50–8:49	.30	.27	.01–1.00
Subjects with dry mouth included ^b												
AM												
1 st test	56	43	77%	.84	.88	0–3.5	4:41	1:00	0–6:30	.21	.24	0–.86
2 nd test	50	38	76%	.69	.85	0–4.4	4:39	1:03	0–8:00	.17	.22	0–.88
PM												
1 st test	47	35	74%	.78	.71	0–2.5	4:15	1:23	0–6:45	.25	.27	0–.89
2 nd test	41	32	78%	.81	.87	0–3.0	4:14	1:23	0–8:49	.24	.27	0–1.00

Note. Data were analyzed with and without those with dry mouth.

^a Dry mouth means no saliva was obtained when attempted; subjects with dry mouth will be excluded from analyses of salivary components.

^b All subjects were included when examining the relationship of saliva variables to medications thought to affect flow rate. Attempts, and success rates were the same in both cases.

Table 3

Saliva Flow Rates in the Literature.

	Simulation	Population	<u>M</u> (ml/min)	<u>SD</u>
Whole saliva (all saliva glands after surgery)				
Karkela et al. 2002	Salivette	Knee surgery	.18	.83
Lahteenmaki et al. 1997	Chewed paraffin	Open heart surgery	.40	.30 to .50
Lahteenmaki et al. 1998	Chewed paraffin	Hysterectomy	.20 to .40	.20 to .70
Smith-Hanrahan, 1997	None	Hysterectomy	.10	.02
Stimulated parotid saliva (both parotid glands after surgery)				
Our study-all subjects	Lemon Juice	Abdominal surgery	.17 to .25	.22 to .27
Our study-no dry mouth	Lemon Juice	Abdominal surgery	.23 to .33	.23 to .27
Stimulated parotid saliva (1 parotid gland in healthy subjects)				
Mandel & Khurana, 1969	.5 % Citric acid	Healthy	.29	.13
"	1% Citric acid	Healthy	.58	.80
"	2% Citric acid	Healthy	.80	.47
Fischer & Ship, 1999	2% Citric acid	Healthy	.24 to .40	.17 to .22