The effect of cigarette smoking on whole stimulated salivary flow rate and pH

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MSc Oral Medicine (2016)

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Declaration

I declare that (The effect of cigarette smoking on whole stimulated salivary flow rate and pH) is my own work and it has not been submitted before for any degree in any other university. And that all the sources I have used or quoted have been acknowledged by references.

Dedication

To my parents for their constant encouragement, support and love

Acknowledgement

I would like to express my gratitude to:

- Dr H Holmes: for her supervision and guidance through the steps of this project.
- Prof LXG Stephen and the staff of the Oral Medicine and Periodontology Department: for their help through my MSc course.
- The staff members in the Sifting and Paedodontic departments as well as the Dental Research Lab at UWC oral health center: for their help during the data collection phase.
- Prof JS Maritz: for the assistance with the data statistical analysis.

Contents

Abstract

Introduction: Saliva is a significant biological fluid involved in the maintenance of good oral health. Cigarette smoking exerts detrimental effects on oral health and has been shown to affect saliva, but with no consensus regarding its effect on the quantity (flow rate) and quality (pH) of the saliva.

Aim: To assess the effect of cigarette smoking on the flow rate and pH of whole stimulated saliva**.**

Method: A case control study was conducted using patients who presented at the UWC Oral Health Centre patient sifting/waiting area. The patients who agreed to participate were assessed for inclusion into the study until the sample size was (n=60), stratified by smoking (n=30) and non-smoking (n=30). Stimulated saliva samples were collected in specimen jars by asking patients to chew a sterilized rubber band for 5 minutes and spit the contents into the specimen jar provided at 1 minute intervals. The specimens were transported to the laboratory within 30 minutes to measure the salivary quantity and pH.

Results: No statistically significant difference in the salivary flow rates was found between smokers and non smokers (p=0.5273). Smokers showed a statistically significant decrease in their pH compared to non smokers (p=0.028).

Conclusion: Cigarette smoking reduces the salivary pH, thereby producing an acidic environment.

Key words: stimulated saliva, cigarette, pH

1- Background and purpose for the study

Tobacco use is a worldwide phenomenon and the World Health Organization (WHO) estimates that about 80% of the world's cigarette smokers live in developing countries. Tobacco users are estimated at being around 1.3 billion people and are expected to rise reaching about 1.6 billion by the year 2030, with tobacco related deaths accounting for 6 million lives per year. (Available from) <http://www.who.int/mediacentre/factsheets/fs339/en/> and <http://www.who.int/oral_health/media/orh_tobacco_fdi_book.pdf>

The effects of cigarette smoking on systemic and oral health are well documented. These include lung cancer, oral cancer, cardiovascular diseases, teeth discolouration, halitosis, salivary gland dysfunction and oral candidiasis (Reibel,2003;Winn, 2001and Johnson and Bain, 2000).

Saliva is a biological fluid of great clinical significance and can be used to study the health status of people, diagnose and monitor systemic and oral disease progression. It is easily accessible, non-invasive and is a cost-effective screening tool (Deepa and Thirrunavukkaarasu**,** 2010 and Brandtzaeg, 2007).The multifactorial roles of saliva (such as protection and lubrication) are needed for the preservation of oral health (Rad *et al*, 2010) and can be determined by assessing the flow rate and pH (Kanwar *et al*, 2013).

Studies evaluating the effect of cigarette smoke on unstimulated saliva have already been carried out with conflicting results (Palomares *et al*, 2004, Kanwar *et al*, 2013 and Dyasanoor and Saddu, 2014). There is a paucity of literature documenting the effect of smoking on stimulated saliva flow rate and pH.

2- Literature review

2.1- Cigarette smoking:

Cigarette smoking is a complex external and internal stimulus consisting of visual, tactile, mechanical (mouth movement); olfactory, gustatory, and irritational factors (Doni *et al*, 2013).The danger of cigarette smoking is masked by the marketing strategies employed by tobacco companies. Regardless of its form or packaging, cigarette smoke contains both highly toxic and carcinogenic chemicals, such as polycyclic aromatic hydrocarbons, aromatic amines and nitrosamines (Winn, 2001) and (Cancer Research UK); (available from) <http://www.cancerresearchuk.org/cancer-info/healthyliving/smoking-and-cancer/whats-in-a-cigarette/smoking-and-cancer-whats-in-a-cigarette>

Cigarette smoking is a great risk factor for lung cancer, cardiovascular diseases and increase in miscarriages and low-birth weight babies in female smokers. In addition wound healing in smokers is delayed when compared to non-smokers (Johnson and Bain, 2000 and Sopori, 2002).

There is a strong association between cigarette smoking and oral mucosal lesions such as smoker's palate, smoker's melanosis, black hairy tongue, periodontal diseases and oral cancer, with tobacco users having increased association compared to non-users (Reibel, 2003and Winn,2001).

2.2- Saliva:

Saliva is a viscoelastic solution comprising 99% water, with proteins and ions making up the remainder. About 0.5-1.0 liter of saliva is produced on a daily basis, 90% of which is produced by the major salivary glands (parotid, submandibular and sublingual) (Figure 1). The minor salivary glands account for the remaining 10% and are scattered throughout the oral mucosa except the dorsum of the tongue, anterior hard palate and the gingivae (Carpenter, 2013; Dawes, 2008and Pedersen, 2007).

The collection of saliva secreted from all salivary glands is called "whole saliva". When secreted under resting conditions, it is referred to as "unstimulated". "Stimulated saliva" is produced following exposure to a stimulus such as chewing or taste and is mainly secreted from the parotid glands (Navazesh and Kumar, 2008 and Carpenter, 2013).The functions of saliva (Figure 2) range from lubrication, to killing microorganisms, to preventing dental caries and to helping with food tasting and digestion (Carpenter, 2013;Dawes, 2008 and Pedersen, 2007).

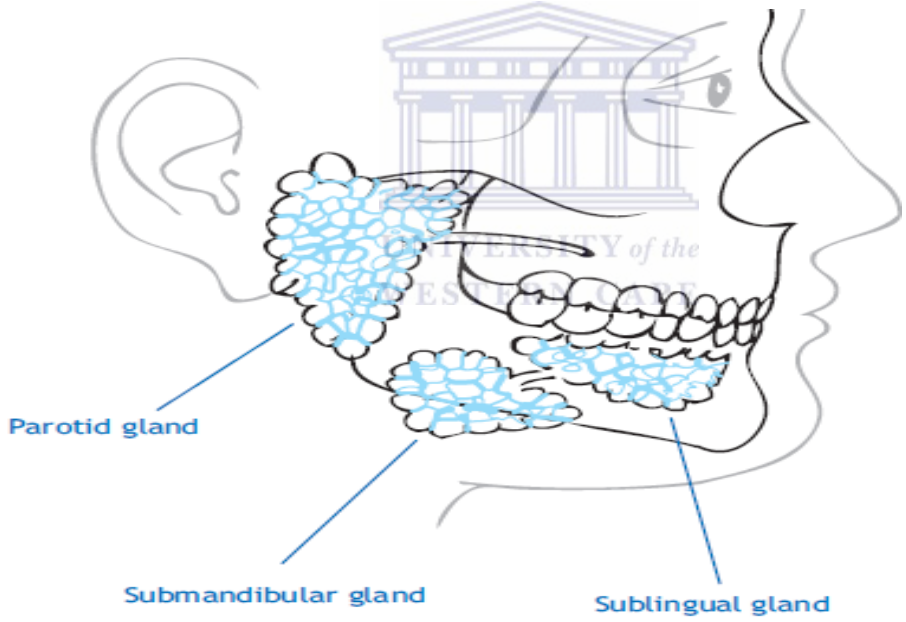


Figure 1: Major Salivary Glands (Pedersen, 2007)

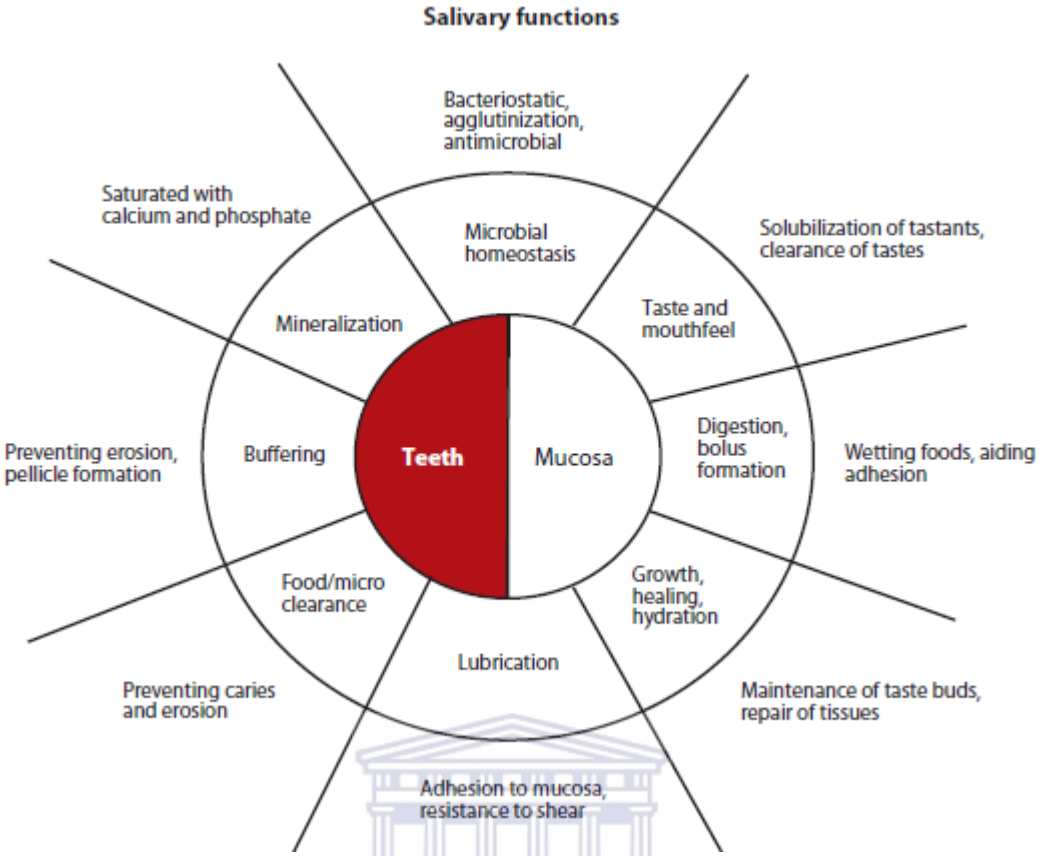


Figure 2: Functions of Saliva (Carpenter, 2013)

The protective function of saliva is attributed to components such as immunoglobulin A, lysozymes and histatins which act as antimicrobials. Water and mucins are necessary for cleansing and lubrication of oral mucosa and teeth. In addition they are responsible for the dissolution of taste compounds and bolus formation. The buffering action of saliva is provided by the bicarbonate, phosphate and protein constituents. The calcium and phosphate ions contribute towards teeth remineralization (Carpenter, 2013; Dawes, 2008 and Pedersen, 2007).

2.3- Salivary Flow Rate and pH:

The salivary flow rate is influenced by the circadian rhythm, implying that it increases during the day and is negligible during sleep. Approximately two thirds of resting whole saliva volume is produced by the submandibular glands (0.3-0.5 ml/min). The parotid glands are responsible for secreting about one half of the stimulated saliva

volume (1.0-1.5 ml/min), after stimulation by taste or chewing (Carpenter, 2013; Dawes, 2008 and Pedersen, 2007).

Parotid saliva contains a high bicarbonate concentration and thereby the salivary pH is correlated with the salivary flow rate. It has been noticed that patients with low flow rate, had lower bicarbonate concentration and salivary pH (Pedersen, 2007 and Palomares *et al*, 2004).

The significance of saliva in the preservation of oral health is apparent when patients complaining of dry mouth present with diseases such as oral candidiasis and mucosal abrasions (Dawes, 2008).This unpleasant sensation of oral dryness is termed (xerostomia) and may negatively affect the patient's quality of life (Dyasanoor and Saddu, 2014).

Since the measurement of salivary flow rate is not routinely performed in dental clinics, there are no baseline data available for comparison. Therefore, commonly the reduction in salivary flow rate which is termed (hyposalivation) is considered when the unstimulated whole saliva flow rate is $\leq$ 0.1 ml/min and the stimulated whole saliva flow rate is $\leq$ 0.7 ml/min (Dyasanoor and Saddu, 2014; Dawes, 2008 and Pedersen, 2007).

The reduction of salivary flow is associated with a variety of medical conditions such as (Sjogren's syndrome, diabetes mellitus, rheumatoid arthritis and Human Immunodeficiency Virus infection (HIV); medications (anti-hypertensives, antihistamines, antidepressants and antipsychotics) and radiation therapy of the head and neck. The use of multiple medications is the most common cause of dry mouth (Dawes, 2008; Navazesh and Kumar, 2008 and Pedersen, 2007).

2.4- The relationship between cigarette smoking, salivary flow rate and pH:

Studies have reported on the association between salivary flow rate and cigarette smoking (Table 1). Smoking was found to produce an initial increase in the stimulated

salivary flow, then no difference found between chronic smokers and non-smokers (Parvinen, 1984; Khan *et al,* 2010 and Johnson and Bain, 2000).

Accordingly an initial increase in salivary pH occurs, with some studies reporting the pH to lowers over time (Johnson and Bain, 2000 and Reibel,2003). Others however reported no variation (Palomares *et al,* 2004). This could probably be due to variations in study designs and methods of investigations implemented by these studies.

Table 1: Studies reporting on the influence of smoking on saliva

Study	**Type of Saliva**	**Test for pH**	**Conclusion**
(Dyasanoor and Saddu,2014)	Unstimulated	No	Significant reduction in salivary flow rate.
(Kanwar *et al,*2013)	Unstimulated	Yes	Significant decrease in salivary flow rate and pH.
(Voelker *et al,*2013)	Unstimulated Stimulated	Yes	Association between stimulated salivary pH and smoking were not statistically significant.
(Rad *et al*, 2010)	Unstimulated	No	Smoking significantly reduces salivary flow rate.
(Khan *et al,* 2010)	Unstimulated Stimulated	No	Smoking not affects the salivary flow rate.
(Palomares *et al,* 2004)	Unstimulated	Yes	Smoking does not imply alterations on salivary flow and pH.

(Parvinen,1984)	Stimulated	Yes	Smoking was not associated with significant changes in salivary flow rate. The pH was lower in smokers.

2.5- Methods for measuring saliva:

Several methods (Table 2) had been implemented to measure the salivary flow rate. Generally to collect whole saliva samples, the participants need to refrain from eating, drinking (except for water) or smoking for about one hour before saliva collection. They should rinse their mouths several times with distilled water. At the beginning of the test the participants should swallow any remaining saliva in their mouths. To test the unstimulated saliva the participant will have to reduce their mouth movements while for stimulated saliva the participants may chew inert chewing gum (Navazesh and Kumar, 2008).

These studies were usually performed in the morning between 9:00 am to 12:00 pm in order to avoid the diurnal variations.

Table 2: Methods for measuring saliva

Study	**Method**
(Dyasanoor and Saddu, 2014)	Unstimulated whole mouth salivary flow, assessed by a test strip placed into the floor of mouth for 3 minutes and readings recorded at minute intervals, which were performed using modified Schirmer test.
(Rad *et al*, 2010)	Whole saliva was collected in the resting condition, by spitting in a graduated container every 1 minute for 5 minutes.
(Kanwar *et al*, 2013)	Saliva was collected under resting condition for 10 minutes. Salivary pH was determined using salivary pH strips.

(Palomares *et al,* 2004)	Unstimulated whole saliva was collected during ten minutes, and salivary flow rate (ml/min), pH, and bicarbonate concentration (mmol/l) were measured using a Radiometer ABL 520.
(Khan *et al,* 2010)	The saliva was collected under resting condition by saliva pool in a graded tube for 10 minutes and following application of crude nicotine and citric acid solution to the tip of tongue.
(Voelker *et al*,2013)	Collection of stimulated saliva samples by chewing a piece of wax; TwinpH electronic meter and pH paper strip were used to measure salivary pH.
(Parvinen,1984)	Paraffin stimulated whole saliva.

Variety of methods are applied to collect saliva and thus the main concern about using and measuring saliva to evaluate many health-related issues is the lack of standardized methods for saliva collection (Navazesh and Kumar, 2008).

3- The Research Design and Methodology

3.1- Aim:

To assess the effect of cigarette smoking on the flow rate and pH of whole stimulated saliva.

3.2- Objectives:

1- To assess the flow rate and pH of stimulated saliva in cigarette smokers and non smokers groups.

2- To compare the difference in the flow rate and pH of stimulated saliva between the two groups.

3.3- Null Hypothesis:

There is no difference in the stimulated salivary flow rate and the pH in smokers versus non smokers.

3.4- Methodology:

3.4.1- Study design:

A case control study was carried out on cigarette smokers (*n*=30) and non smokers (*n*=30).

3.4.2- Inclusion criteria:

1-Apparently healthy volunteers, age 18-45 years, with no medications or medical conditions affecting saliva secretion, or had head and neck radiation therapy.

2- Cigarette smokers who have been smoking a minimum of 5 cigarettes a day for 6 months.

3.4.3- Exclusion criteria:

1-Cigarette smoker of less than 5 cigarettes a day or for period that is less than 6 months.
2- Participant who diagnosed with Sjögren's syndrome, diabetes mellitus, rheumatoid arthritis or HIV.
3- Participant who is on antihypertensive, antihistamines, antidepressants or antipsychotic medications.
4- Participant who had head and neck radiation therapy.

3.4.4- Sample size:

According to statistical calculation 30 participants were enrolled in each group. This would ensure that the difference between means of groups (Δ) =0.25; a standard deviation of (σ) =0.20 for the salivary flow rate between subjects and the significance level (p) </=0.05.

3.4.5- Study Site and Sample selection procedure:

Participants were selected from consecutive adult persons presenting at the waiting area of the Sifting Department at the UWC Oral Health Center (Tygerberg Campus).
Persons who met the inclusion criteria and were willing to participate were included in the study until the required number for each group was attained.
All participants were briefed about the study, and informed consents were obtained.

3.4.6- Data collection time:

Saliva sample collection was obtained in the morning to avoid diurnal variation.

3.4.7- Material:

The following materials were used:

- Pieces of inert chewing material (rubber bands) to stimulate salivary secretion.
- Gas sterilizer to sterilize the rubber bands.

- Stopwatch to alert the participants to spit at every one minute.
- Specimen bottles were used for saliva samples collection.
- And Beckman pH meter.

3.4.8- The Spitting Method was used for sample collection:

Steps of saliva sample collection:

All participants were advised to:

1- Avoid eating, drinking (except for water) or smoking for one hour before the start of the test.

2- Rinse their mouths with water several times (minimum 3 times) at the beginning of the test.

3- Swallow any remaining saliva prior to taking sample collection.

4- Chew the rubber band to stimulate salivary secretion and spit in the specimen bottle at every one minute for five minutes.

5- Avoid speaking or swallowing during the test.

3.4.9- The salivary flow rate:

It was measured in (ml/min) from the volume of saliva in the specimen bottle after 5 minutes.

The saliva samples were transferred from the specimen bottles to the test tubes using the Pipetman® pipettes, and for each sample a new disposable tip was used.

3.4.10- The salivary pH:

It was measured by the Beckman pH meter.

Measurement of pH was performed within half an hour following saliva collection, and the Beckman pH meter was calibrated every morning.

3.5- Data analysis:

The collected data readings were recorded in a data collection Excel® spread sheet and analyzed by a statistician using the same program.

3.6- Ethical considerations:

The study was carried out after obtaining ethical approval from the Research Ethics Committee of the University of the Western Cape.

The study procedure was non invasive and not harmful to participants and the confidentiality of participants was preserved.

Informed consents were obtained from all the participants after explaining the aim of the study and the method of its application.

3.7- Budget:

All the study requirements were available from the Dental Research Lab at UWC.

4-The Results

The data were analyzed by the use of Kruskal-Wallis test, i.e. the one way analysis of variance test, i.e. ANOVA.

Table 3: The Data Table

Number	Age	Sex	Smoking	Cigarette/day	Saliva (ml) in 5 min	Saliva (ml/min)	pH
1	38	male	yes	7	5.6	1.12	7.34
2	25	male	yes	15	14	2.8	7.12
3	45	male	yes	7	4.6	0.92	7.50
4	45	male	yes	10	3.7	0.74	6.76
5	42	male	yes	10	0.5	0.1	7.70
6	25	male	yes	20	2	0.4	7.32
7	24	female	yes	10	7.3	1.46	7.44
8	25	female	yes	5	2.6	0.52	7.09
9	20	male	yes	7	5	1	7.15
10	35	female	yes	6	2.3	0.46	6.81
11	28	female	yes	7	3.6	0.72	7.07
12	22	male	yes	10	5.1	1.02	7.63
13	22	female	yes	10	4.2	0.84	7.32
14	27	female	yes	10	3.8	0.76	6.76
15	29	male	yes	8	5.8	1.16	7.45
16	25	female	yes	8	3.2	0.64	7.68
17	24	male	yes	10	5.1	1.02	7.39
18	29	male	yes	20	3.2	0.64	7.83
19	39	male	yes	30	17	3.4	7.04
20	30	male	yes	10	7.5	1.5	7.40
21	36	female	yes	10	5	1	6.90
22	43	male	yes	10	6.8	1.36	7.53
23	41	male	yes	15	5	1	7.09
24	33	male	yes	15	4.7	0.94	7.56
25	34	female	yes	10	2.8	0.56	7.76

26	25	female	yes	20	4.4	0.88	7.35
27	28	female	yes	15	2.3	0.46	6.96
28	27	male	yes	28	4.2	0.84	7.39
29	24	male	yes	15	2.7	0.54	6.83
30	22	male	yes	6	5.8	1.16	7.18
31	39	female	No	0	1.6	0.32	7.04
32	20	female	No	0	2.2	0.44	7.15
33	26	female	No	0	2	0.4	7.01
34	31	male	No	0	9.2	1.84	7.60
35	19	female	No	0	2.4	0.48	7.44
36	30	male	No	0	4.4	0.88	7.60
37	38	female	No	0	1.8	0.36	7.85
38	43	male	No	0	1.5	0.3	7.71
39	43	female	No	0	19	3.8	7.66
40	35	female	No	0	5.8	1.16	7.44
41	30	female	No	0	3	0.6	7.23
42	29	male	No	0	7.2	1.44	7.96
43	26	female	No	0	1.7	0.34	7.56
44	27	male	No	0	7.6	1.52	7.56
45	35	female	No	0	6.6	1.32	7.39
46	34	female	No	0	13	2.6	7.72
47	40	female	No	0	6.3	1.26	7.63
48	22	female	No	0	4.6	0.92	7.34
49	30	female	No	0	4.5	0.9	7.59
50	39	female	No	0	3.6	0.72	7.32
51	20	female	No	0	8.3	1.66	7.37
52	25	male	No	0	3.8	0.76	7.48
53	43	female	No	0	2.7	0.54	7.92
54	26	male	No	0	4.2	0.84	7.36
55	22	female	No	0	4.8	0.96	7.06
56	37	female	No	0	5	1	7.67
57	26	female	No	0	7	1.4	7.33
58	41	female	No	0	5	1	8.03
59	25	male	No	0	5.4	1.08	7.17
60	19	female	No	0	4	0.8	7.33

Table 4: Sample size

	Smokers	Non smokers	Total
Male	19	8	27
Female	11	22	33
Total	30	30	60

Table 5: Smokers group

Cigarette/day	5-10	11-15	≥16	Total
Male	11	4	4	19
Female	9	1	1	11
Total	20	5	5	30

Table 6: The Age statistics

	Number		Mean		Std.Dev.*	
Smoking	No	Yes	No	Yes	No	Yes
Female	22	11	31.09	28.09	8.43	4.78
Male	8	19	29.50	31.74	5.90	8.63

Std.Dev* (standard deviation)

4.1- Salivary flow result:

There is no statistical significant difference in the flow rate between smokers and non-smokers, (p=0.5273).

Table 7: Salivary flow (ml/min) statistics

	Number		Mean		Std.Dev*	
Smoking	No	Yes	No	Yes	No	Yes
Female	22	11	1.045	0.755	0.814	0.294
Male	8	19	1.083	1.140	0.494	0.771

Std.Dev* (standard deviation)

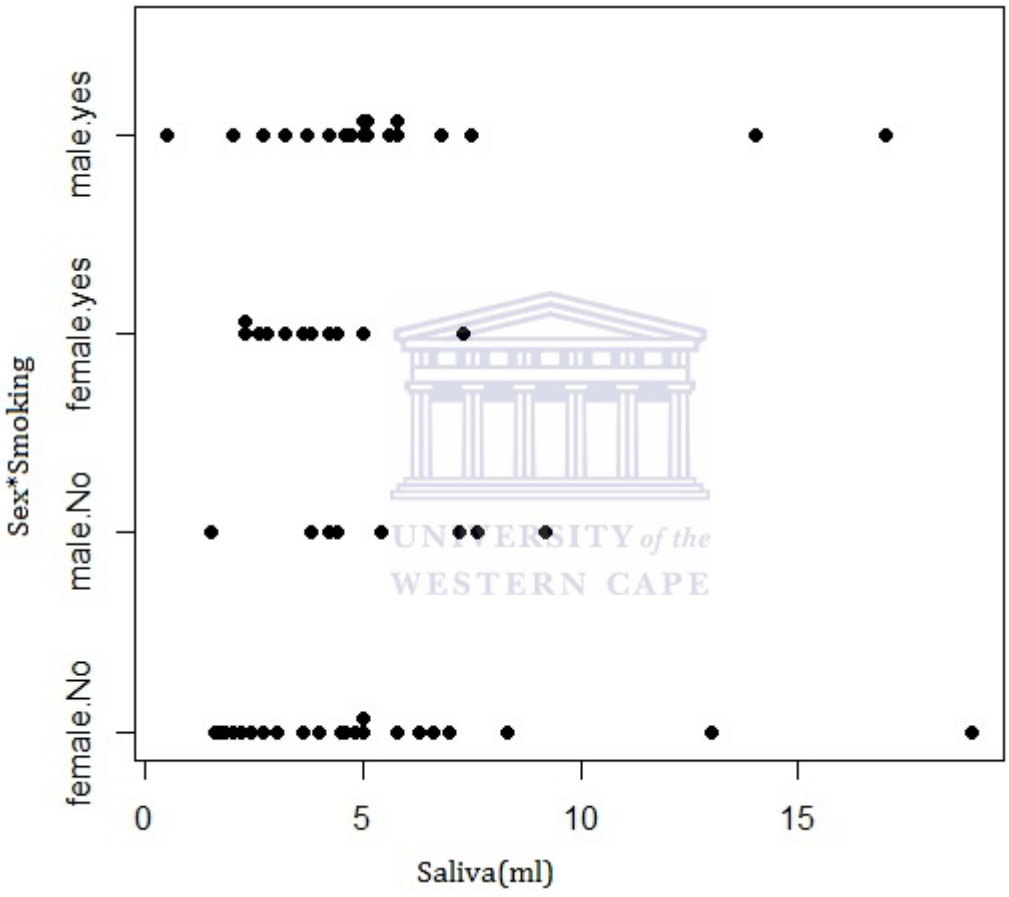


Figure 3: saliva by sex and smoking group

4.2- pH result:

There is statistical significant difference in pH between smokers and non-smokers, (p=0.028).

Smokers were found to have a lower pH than non-smokers.

Table 8: pH statistics

	Number		Mean		Std.Dev	
Smoking	No	Yes	No	Yes	No	Yes
Female	22	11	7.458	7.195	0.282	0.341
Male	8	19	7.555	7.327	0.234	0.283

Std.Dev* (standard deviation)

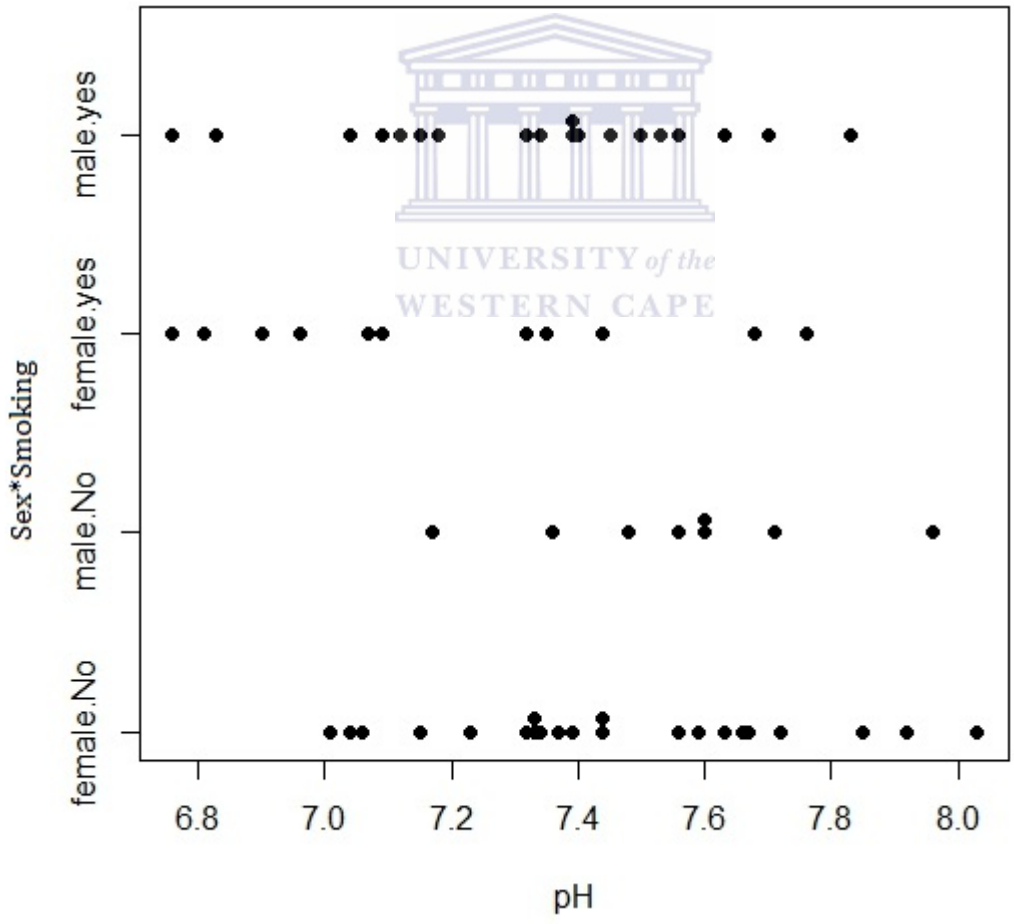


Figure 4: pH by sex and smoking groups

Table 9: Hyposalivation

	Non smoker	Smoker	Total
Female	8	5	13
Male	1	4	5
Total	9	9	18

An incidental finding noticed among the results was the hyposalivation (p=0.283), which is measured when the stimulated whole saliva flow rate is ≤ 0.7 ml/min (Dyasanoor and Saddu, 2014;Dawes, 2008 and Pedersen, 2007).

5- Discussion

The purpose of the study was to evaluate the effect of cigarette smoking on whole stimulated salivary flow rate and pH.

The results of the study showed only a marginal difference between the stimulated salivary flow rate of smokers and non smokers, with no statistically significant differences between the two groups. Parvinen, 1984 and Khan *et al*, 2010 found the same results in their studies even though there were variations in the sample size of these two studies. Parvinen, 1984 had a larger sample size, but the control group was smaller. Khan *et al*, 2010 had a smaller sample size, with an equal number of control participants. Our findings are supported by a study by Johnson and Bain, 2000 who found that regular smokers develop a tolerance following the initial increase seen in salivary flow rate in first time smokers.

This study shows that the mean of the stimulated salivary pH of smokers was statistically significant and lower than that of the non smokers and this was also similar to study done by Parvinen, 1984. Normally the salivary flow rate and pH are directly proportional, but here there was a reduction in the salivary pH of smokers despite the fact that the salivary flow rate of the two groups was equal ; this could be related to factors other than the flow rate such as the effect of cigarette smoking on the chemical composition of the saliva such as bicarbonate (HCO_3) concentration or the influence of the smoke heat on saliva .On the other hand, Voelker *et al*, 2013 found no significant association between cigarette smoking and the stimulated salivary pH. Moreover other contributing factors such as caries risk were examined**.**

The finding of this study can be interpreted clinically, where despite the fact that there is no difference in the quantity of the saliva (flow rate) between the cigarette smokers and non smokers; Smokers had a lower pH value. This acidic oral environment would create favorable bacterial conditions to produce halitosis (oral malodor), and impair the functions of the saliva such as buffering action, teeth remineralization and proper tasting.

The salivary flow rate and pH are usually correlated, as shown by Pedersen, 2007 and Palomares *et al,* 2004. Both studies found that patients with a low flow rate had lower bicarbonate concentration and therefore a lower salivary pH. This was contrary to the findings of our study. It may thus be necessary to examine and compare the chemical composition (e.g. bicarbonate concentration) of smokers and non smokers.

Even though none of the 60 participants had a known medical condition which could affect salivary secretion, 30% (18/60)were found to have hyposalivation (p=0.283), which was an incidental finding manifested in both sexes and half of them were smokers (n=9), and therefore it was not related to neither the sex nor smoking status of the participant. This may have been influenced by other factor such as stress**.**

6-Conclusion

Cigarette smoking is a complex stimulus which has deleterious effects on oral health, ranging from reversible conditions such as halitosis to life-threatening diseases such as squamous cell carcinoma. Various studies have been conducted to its effect on the saliva which is a significant biological fluid, but conflicting results were found. This study shows that even though cigarette smoking did not affect the stimulated salivary flow rate, it reduced the salivary pH.

7- Recommendation

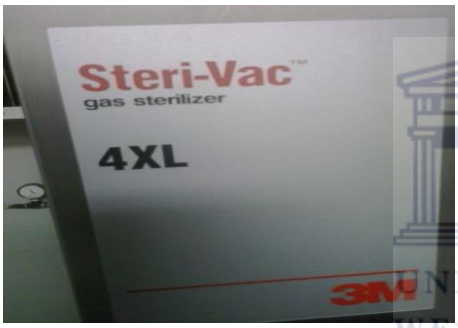
Further studies investigating factors which could influence the stimulated salivary flow rate and pH are required to confirm the above mentioned observations. These include factors such as dentate status, prosthodontic prosthesis, caries risk, periodontal disease, soft tissue infections such as *Candida* and stress. In addition, comparison of salivary flow rate and pH in persons who smoke and could be encouraged to quit smoking would be valuable.

Appendix 1

The equipment

Beckman pH meter

Rubber bands

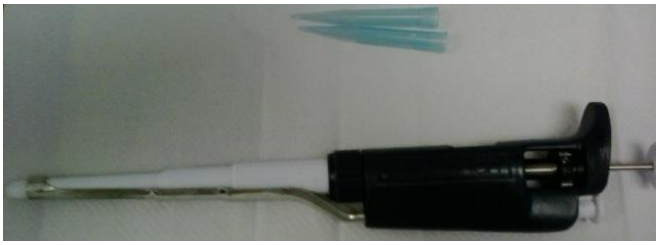


Gas sterilizer

Specimen bottle

Stopwatch

Test tube



Pipetman® and disposable tips

Appendix 2

Questionnaire

Please answer the following questions:

Age: ----- Years Sex: Male ----- Female -----

Smoking Habit: Non-smoker: ----- Smoker: -----

If smoker:

Number of cigarettes smoked a day: -----

Period of smoking: Months: ----- Years: -----

Are you suffering from?

Allergies Yes ---- No ----

Hypertension Yes ---- No ----

Diabetes mellitus Yes ---- No ----

Sjögren's syndrome Yes ---- No ----

Arthritis Yes ---- No ----

HIV Yes ---- No ----

Other ----------

Have you had a radiation therapy to Head and Neck?

Yes ---- No----

Are you taking any medications?

Yes ----- No ----

If "Yes" specify: ----------

Appendix 3

INFORMED CONSENT

Good day

I am Dr Noha Gadour and I am a dentist carrying out a research project for a Master's degree in the Department of Oral Medicine & Periodontology, at the University of the Western Cape Dental Faculty.

I would like for you to take part in my study, which will try to find out if cigarette smoking has any effect on the flow rate and pH of your saliva. This will be done by asking you to answer a few questions, followed by collecting saliva in a tube.

All information obtained will be strictly confidential and your name will not appear on the form or the specimen jar in which your saliva is collected. You are completely free to decide whether to take part in the study or not and your decision will not negatively impact on you receiving treatment at this facility.

Sincerely

Dr Noha Gadour

===

I accept that the purpose and procedure of this study has been explained to me and that I agree to take part in it.

I also understand that enrollment in the study will be anonymous and that the results will be published for the benefit in the medical/dental field.

Name: Signature: Date:

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