

https://dspace.org/

Buffering capacity of saliva, salivary flow rates and cortisol levels in patients with active caries

Hira, Priyesh Gunvant

2013-03-18
http://hdl.handle.net/10539/12545

Downloaded from a DSpace repository. https://dspace.org/

BUFFERING CAPACITY OF SALIVA, SALIVARY FLOW RATES AND CORTISOL LEVELS IN PATIENTS WITH ACTIVE CARIES

P G Hira

Johannesburg

2012

BUFFERING CAPACITY OF SALIVA, SALIVARY FLOW RATES AND CORTISOL LEVELS IN PATIENTS WITH ACTIVE CARIES

Priyesh Gunvant Hira

A research report submitted to the School of Oral Health Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Dentistry.

Johannesburg

2012

Declaration

I, Priyesh Gunvant Hira, declare that this research report is my own work. It is being submitted for the degree of Master of Science in Dentistry in the University of Witwatersrand, Johannesburg. It has not been submitted or incorporated before in another research report, dissertation or thesis for another degree or examination at this or any other University. The experimental work was performed in the Department of Oral Microbiology, School of Oral Health Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg.

_____

P G Hira

12th day of November, 2012

Dedication

This research report is dedicated to my parents, Gunvant and Kanta, and my brothers, Yatin and Bhavik.

Acknowledgements

For the tireless efforts of Professor Maeve M. Coogan whose guidance during the experimental phase and advice in preparing this research report were of immense value. For grants from the Medical Faculty Research Endowment Fund, Faculty of Health Sciences, University of the Witwatersrand and the South African Dental Association (SADA) which are most appreciated.

Abstract

Introduction: Dental caries is caused by the interaction of the host, oral flora and diet. Stress is one of the host factors implicated. Studies have shown that there is an association between stress and salivary cortisol levels. However, no studies have investigated the relationship between stress, salivary cortisol levels and caries susceptibility. **Aims and objectives:** The aim of the study was to determine whether there is a correlation between active dental caries, resting and stimulated flow of saliva, salivary buffering capacity, saliva cortisol levels and stress in patients attending a general dental practice in Lenasia South. **Methods and materials:** Sixty subjects between the ages of 18 and 60 were included in the study. Thirty controls with no active caries, a minimum of 28 teeth and a mean decayed, missing filled surfaces (DMFS) score of 4 or less, and 30 subjects with active caries were included in the study and formed the experimental group. Patients with Sjögren's Syndrome or connective tissue diseases, on medication that may cause xerostomia, or a history of previous or current irradiation were excluded from the study. At the initial visit resting and stimulated saliva samples were collected and the volume was measured. The buffering capacity and cortisol levels of the resting saliva samples were measured. In addition the Depression Anxiety Stress Scales (DASS) questionnaire was used to determine the stress levels of the participants. The teeth of the subjects with active caries, i.e. the experimental group, were restored. They returned after 4 weeks for a follow up visit and their resting and stimulated salivary flow, buffering capacity of saliva, salivary cortisol and the stress levels were measured. The results were compared using the two sample t test, chi – squared test and a generalized logistic regression analysis. **Results:** The DMFS of the control group, 0.40 ± 0.97, was significantly lower ($p < 0.001$) than 29.27 ± 21.94, in the experimental group. No significant differences were found between the controls and caries prone subjects when the resting flow rates, 0.37 ± 0.30 ml/min and 0.32 ± 0.19 ml/min; stimulated flow rates, 0.99 ± 0.56 ml/min and 0.84 ± 0.35 ml/min; buffering capacity of saliva, 19.16 ± 4.68 ml 0.01N lactic acid and 21.73 ± 9.77 ml 0.01N lactic acid, were compared and the salivary cortisol levels of the controls 17.71 ± 22.51 ng/ml, were higher than 11.80 ± 14.61 ng/ml in the the caries prone subjects. The DASS scores of the two groups were similar, i.e. 11.33 ± 8.48 and 11.2 ± 9.6, respectively. After the carious teeth of the caries prone subjects were restored, the flow rate of resting saliva increased from 0.32 ± 0.19 ml/min to 0.37 ± 0.16 ml/min, the stimulated saliva from 0.84 ± 0.35 ml/min to 0.88 ± 0.32 ml/min and the buffering capacity of saliva from 21.73 ± 9.77 ml 0.01N lactic acid to 22.25 ± 7.55 ml 0.01N lactic acid and the salivary cortisol levels decrease from 11.80 ± 14.61 ng/ml to 10.00 ± 12.12 ng/ml. Again none of these differences were significant. **Conclusion:** These results suggest that stress levels measured by the DASS questionnaire may not be related to caries. A less subjective questionnaire may find a relationship between salivary cortisol levels, stress and dental caries.

Table of Contents

Declaration..... iii

Dedication..... iv

Acknowledgements..... v

Abstract..... vi

Table of Contents..... vii

List of Figures..... ix

List of Tables..... ix

Chapter 1: Introduction..... 1

Chapter 2: Literature review..... 4

2.1. Caries and salivary flow rate..... 4

2.2. Caries and buffering capacity of saliva..... 5

2.3. Salivary flow rate and buffering capacity..... 6

2.4. Salivary cortisol, dental caries and salivary flow rate..... 6

2.5. Stress and salivary cortisol levels..... 6

2.6. Measurement of stress..... 9

Chapter 3: The present study..... 10

3.1. Aims and Objectives..... 10

Chapter 4: Methods and Materials..... 11

4.1. Selection of patients..... 11

4.1.1. Experimental group..... 11

4.1.2. Control group..... 11

4.2. Clinical caries examination..... 12

4.3. Salivary flow rates..... 12

4.4. Buffering capacity of saliva..... 12

4.5. Salivary cortisol level..... 12

4.6. Measurement of stress using DASS – 42..... 14

4.7. Statistical analysis…… 14

Chapter 5: Results..... 15

5.1. Decayed Missing Filled Surfaces index (DMFS)..... 15

5.2. Salivary flow rates..... 15

5.3. Buffering capacity of saliva..... 16

5.4. Salivary cortisol..... 16

5.5. Depression Anxiety Stress Scales score (DASS)..... 17

Chapter 6: Discussion..... 21

6.1. Decayed Missing Filled Surfaces index (DMFS)..... 21

6.2. Salivary flow rates..... 21

6.3. Buffering capacity of saliva..... 21

6.4. Salivary cortisol and Depression Anxiety Stress Scales score (DASS). 22

6.5. Limitations of the current study…… 23

Chapter 7: Conclusion..... 24

Appendix A: List of abbreviations used in the text and tables..... 26

Appendix B: Ethics clearance certificate..... 27

Appendix C: Forms for participants..... 30

Information sheet and consent form..... 31

DASS questionnaire..... 33

Appendix D: Analyses recording forms..... 35

Chapter 8: List of references..... 37

List of Figures

Figure 1.1 – The 3 circles represent the interplay of the aetiological factors in dental caries. All 3 factors must be acting simultaneously for caries to occur..... 1

Figure 2.1 – Assembly and components of Forsite Diagnostics competitive lateral flow assay..... 8

Figure 5.1 – The number of control and experimental subjects with normal, mild, moderate, severe and extremely severe stress levels at the initial visit and stress levels in the experimental subjects one month later..... 18

List of Tables

Table 5.1 – The demographic characteristics and saliva flow, buffering capacity, salivary cortisol, Depression Anxiety Stress Scales score and DMFS of the control group, experimental group and experimental group at the 1 month follow up visit..... 19

Table 5.2 – Correlation matrices for comparison of different variables..... 20

Chapter 1

1. Introduction

Dental caries is the most common chronic oral disease in mankind. Regardless of gender, socioeconomic strata, race and age, all people are affected[1].

For caries to develop, the presence and interaction of three main factors is essential, namely the host and teeth, the presence of microorganisms and the host's diet. The integration of these factors results in the occurrence of caries[2]. Dental caries is a disease which causes localized destruction of the mineralized tissues of the teeth, namely, enamel, dentine and cementum, that results from the action of microorganisms on fermentable carbohydrates. Caries is also affected by oral hygiene and saliva[1]. Characteristically the mineral component of teeth is demineralised and thereafter disintegration of the organic matrix occurs[2, 3].

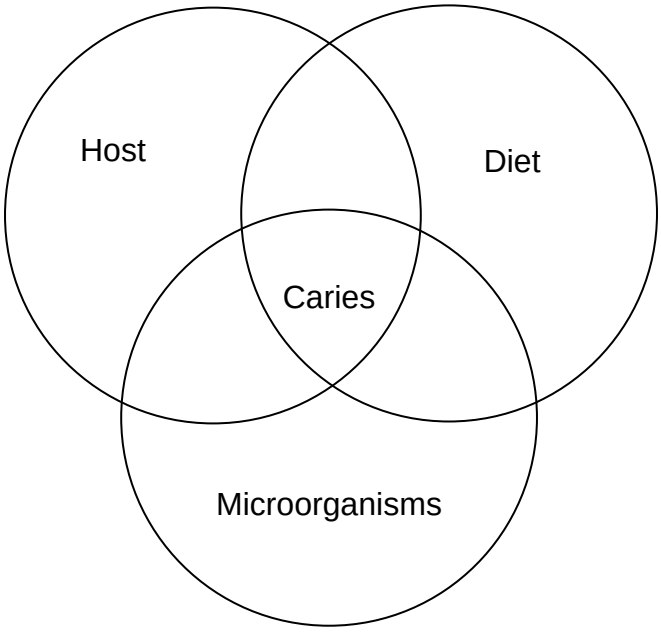


Figure 1.1 – The 3 circles represent the interplay of the aetiological factors in dental caries. All 3 factors must be acting simultaneously for caries to occur[2].

The microorganisms and their products form an adherent deposit, namely, dental plaque, which is found on all tooth surfaces. Initially dental plaque contains a large proportion of streptococci which grow, multiply and produce an extracellular polysaccharide which is sticky and subsequently traps other bacterial forms. As a result the microbial flora of plaque changes from mainly cocci to a mixed microbial flora of cocci, rods and filaments[2]. The microorganisms implicated in dental caries are *Streptococcus mutans*, *Lactobacilli* and *Actinomycetes*. The cariogenicity of *Streptococcus mutans* and *Lactobacilli* is due to their ability to rapidly produce acid from fermentable carbohydrates[2, 3].

In order for acid to form and cause enamel demineralization the presence of fermentable carbohydrates and plaque on the tooth surface for a minimum length of time is essential. The carbohydrates are utilized by the bacteria to produce acid and extracellular polysaccharides. The cariogenicity of carbohydrates varies with low molecular weight carbohydrates such as sucrose being more cariogenic because they are quickly metabolized to acid by the bacteria. Demineralization of the tooth occurs when the plaque pH is kept depressed by the repeated and frequent consumption of sugar[2].

The susceptibility of the host to caries is dependent upon tooth morphology and the environment of the tooth. Due to tooth morphology the occlusal surfaces of the teeth are prone to caries because these sites favour stagnation and encourage plaque retention. The environment of the tooth is also influenced by saliva[2]. Several studies have identified salivary flow rate and buffering capacity as predisposing factors for the development of dental caries[4]. In addition the production of saliva which constantly bathes the teeth and oral mucosa as well as the protective properties of this fluid, for example, the mechanical washing action, antimicrobial activity, the remineralisation of early carious lesions due to a high concentration of calcium and phosphate ions, buffering capacity, salivary clearance and action as a lubricant prevents the development of dental caries[1, 2, 5, 6].

There are several methods of measuring stress including Lipp's Stress Symptoms Inventory, the Perceived Stress Questionnaire (PSQ), the Perceived Stress Scale (PSS), the Index of Clinical Stress (ICS), the Stress Response Inventory (SRI), the

Trier Inventory for the Assessment of Chronic Stress (TICS) and the Depression Anxiety Stress Scales (DASS) questionnaire[7]. The Depression Anxiety Stress Scales questionnaire was used because it is simple and can be used to measure psychological stress levels which occur when the inability to cope with a challenge is perceived by the mind[8].

The aim of this study was to compare the flow rate of resting and stimulated saliva, buffering capacity, stress levels and cortisol levels of resting saliva from 30 patients with active caries and 30 caries free controls and to determine whether there is a correlation between caries susceptibility, salivary cortisol levels and stress.

Chapter 2

2. Literature review

2.1. Caries and salivary flow rate

Reports on the beneficial effect of saliva flow rate vary. Several studies have found that the protective benefits of saliva such as salivary clearance, buffering capacity and degree of saturation of tooth minerals are increased and maximized upon salivary stimulation[5, 9, 10]. Reduced salivary secretion and increased caries is often found in patients following radiation therapy to the head and neck region, after use of medication which causes hyposalivation, in patients with substance abuse, psychogenic disorders and suffering from Sjögren's syndrome[5, 11, 12]. Stensson, Koch, Oldaeus, Lingström and Birkhed[13] reported that asthmatic subjects with a high caries experience had poor stimulated salivary flow rates compared to a group of age matched healthy controls while Coogan, Mackeown, Galpin and Fatti[14] found that dental subjects with minimal caries experience had high stimulated salivary flow rates. Furthermore, Stookey[15] found that chewing gum after a meal resulted in a significant decrease in the incidence of dental caries due to stimulatory effect on salivary flow rates.

In contrast Akpata, Al – Attar and Sharma[11]; Moritsuka, Kitasako, Ikeda, Nomura and Tagami[16]; and de Castilho, Pardi and Pereira[17] found no significant association between dental caries and salivary flow rate. Caries prevalence in patients with Down's Syndrome did not significantly correlate with their salivary flow rate[17] whereas Akpata *et al*[11] showed that the difference between the stimulated salivary flow rates in patients with severe caries and controls did not reach statistically significant levels. However, Watanabe, Mizoguchi, Masamura and Nagaya[18] found no relationship between salivary flow rate and the DMFT in healthy children. Mass, Gadoth, Harell and Wolff[19] found that increased salivary flow rates in children with Familial Dysautonomia due to salivary gland hyperfunction was a "caries protective" parameter in contrast to children with active caries who had significantly lower

salivary flow rates. Furthermore, Flink[12] reported that reduced salivary flow rates in young adult women were related to their caries experience.

2.2. Caries and buffering capacity of saliva

Reports on caries, saliva flow and buffering capacity vary. For example de Castilho *et al*[17] found that caries prevalence in Down's Syndrome subjects did not significantly correlate with their buffering capacity of saliva. Akpata *et al*[11] showed that the difference between the buffering capacity of saliva in patients with severe caries and controls did not reach statistically significant levels whereas Stensson *et al*[13] reported no significant difference in the buffering capacity of asthmatic patients compared to a group of age matched controls.

In contrast a significantly lower caries experience was observed in patients with Turner's Syndrome who had a high buffering capacity compared to age matched controls[20]. Preethi, Pyati and Dodawad [1] reported that both the buffering capacity and salivary flow rate were reduced in children with active caries compared to caries free children, however, these differences were not significant. Malekipour, Messripour and Shirani[6] found that the pattern of titration of saliva of patients with active caries differed significantly from caries free patients. Joeng, Apostolska, Jankulovska, Angelova, Nares, Yoon, Lim, Angelov and Jeong[21] showed that buffering capacity was very low in subjects with caries compared to the control group who had a very high buffering capacity. However, a significant inverse association has been demonstrated between caries experience and buffering capacity[22]. Furthermore, Coogan and Motlekar[23] and Kinirons[24] found poor buffering capacity is associated with increased caries experience. Kinirons[24] found that patients with cystic fibrosis had lower caries experience and significantly higher buffering capacity than unaffected controls. In addition, enlargement of the submandibular glands and changes in the sublingual and labial mucous glands have been observed in these patients. These changes have been linked to elevated levels of sodium, chloride, calcium, phosphorous, protein, glycoprotein urea and uric acid in saliva[24]. However, functional studies regarding salivary flow rates and buffering capacity of saliva of patients with cystic fibrosis have yielded inconsistent results[25].

In a recent study Mentz[26] compared 20 restored patients with numerous crowns and bridges and 20 control subjects with no crowns. He found no significant differences between the salivary flow rates of fixed prosthodontic patients and the controls. However, after exposure to citric acid the buffering capacity of saliva of the restored patients was significantly lower than the controls ($p = 0.029$). He found that the poor buffering capacity of saliva of his patients had a significant impact on their DMFS and suggested that this may be related to stress because 12 of the 20 restored patients were professional people or company owners who had demanding life styles.

2.3. Salivary flow rate and buffering capacity

Muerman and Rantonen[27] have shown that patients on medication for mental disorders, hypertension, pain, respiratory disorders, metabolic disorders and gastrointestinal disorders had lower salivary flow rates and poorer buffering capacity than patients not taking medication. Wikner[28] found poor salivary flow rates may predict poor buffering capacity. Moritsuka *et al*[16] reported that patients with a good buffering capacity had associated high salivary flow rates while another study found that there was an increase in buffering capacity as the salivary flow rate increased[6].

2.4. Salivary cortisol, dental caries and salivary flow rate

Rai, Hegde, Shetty and Shetty[4] showed there was a significant increase in salivary cortisol levels in children with rampant caries which gradually decreased over three months following dental treatment. Boyce, Den Besten, Stamperdahl, Zhan, Jiang, Adler and Featherstone[29] observed the highest rates of dental pathology among children with a combination of elevated salivary cortisol expression and high counts of cariogenic bacteria whereas, Shigeyama, Ansai, Awano, Soh, Yoshida, Hamasaki, Kakinoki, Tominaga, Takahashi and Takehara[30] found a significant association between salivary cortisol and reduced salivary flow rates and reported that patients with xerostomia had significantly high salivary cortisol levels.

2.5. Psychological stress and salivary cortisol levels

Stress can be defined as persistent tension, irritability and low threshold to becoming upset or frustrated[31]. Several studies have reported associations between stress and salivary cortisol[32, 33, 34, 35, 36]. Raised salivary cortisol levels have been found in

women with a life time diagnosis of Post-Traumatic Stress Disorder, Temporomanibular Disorders, in late pregnancy and during academic examinations [33, 34, 36, 37]; in students before an examination and in response to presentations, fasting and competitions[35, 38, 39]; in normal people on work days compared to weekends[40], during a stressful week[41], in response to performing a stressful task[42] and after viewing a stressful video[43]; in people of low socio – economic status associated with a high perceived stress index[32]; in children during restorative dental treatment[44]; in sports coaches[45] and under acceleration stress[46].

There are several advantages in investigating salivary cortisol because it can be measured frequently[32, 47], the method is not complicated[47, 48], noninvasive [40, 49], stress free[49, 50], not painful[40, 50], does not require trained medical personnel[40, 49] and the test involves direct measurements[51]. Furthermore tests can be repeated at frequent intervals[40, 43, 49], samples do not need special treatment and remain stable at room temperature for up to 7 days[40].

There are several methods available for measuring salivary cortisol, for example the ELISA extraction – free technique[52], Dissociation – enhanced kanthaside fluoroimmunoassay (DELFIA)[53], nano – linker chemistry coupled with surface plasmon resonance detection[49], liquid chromatography – tandem mass spectrometry (LC – MS/MS)[50], enzyme immunoassay[34], flow – filtered portable surface plasmon resonance[48] and automated – in tube – solid phase microextraction (SPME) coupled liquid chromotography – mass spectrometry (LC / MS)[51]. More recently Forsite Diagnostics developed the competitive immunochromatographic lateral flow assay which is the reverse of the well – known home pregnancy test. The Forsite Diagnostics competitive lateral flow assay functions on the principle of competition for binding sites on sensitized latex particles. Polyclonal or monoclonal antibodies raised to salivary cortisol are bound by passive or covalent means to dyed latex particles. The sensitized latex particles are applied onto a release pad by an immersion procedure to produce a stable particle reservoir for release onto a nitro-cellulose-based membrane. The release pad and membrane together with an absorbent pad are assembled into a plastic housing as illustrated below[54]. Two lines of reagents are immobilised on the membrane. The T or test line consists of an antigen or conjugate of salivary cortisol whereas the control line is a line of anti-

species antibody. Results which appear as a single line are positive whereas two lines are negative. Furthermore the intensity of the T or Test line decreases in intensity as the concentration of salivary cortisol increases.

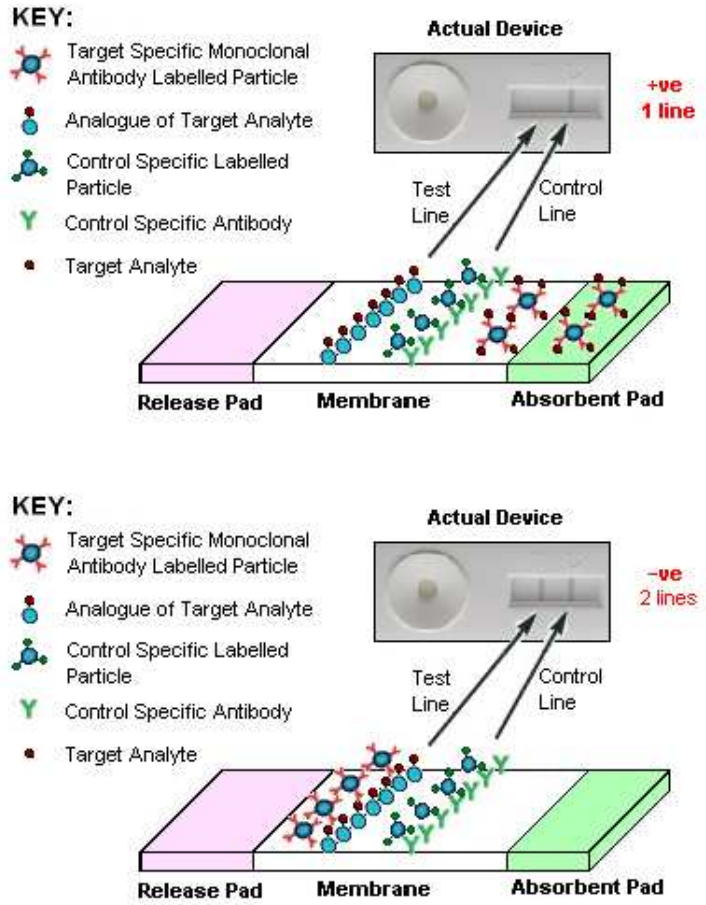


Figure 2.1 – Assembly and components of Forsite Diagnostics competitive lateral flow assay[54].

The plastic cassette housing the membrane test strip had a viewing window which enabled the operator to see the liquid start flowing across the aperture in 20 – 30 seconds.

The application of saliva to the well, releases the latex particles, which then begin to flow across the membrane. The presence of salivary cortisol results in antibody binding producing a latex – antigen complex. Sensitised latex particles that do not bind to salivary cortisol attach to the immobilised T or test line as they traverse the membrane, producing a visible line of deposited latex. A visual confirmation of latex flow is provided when the anti – species antibody captures excess sensitised latex

particles to produce an internal control line. The presence of sufficient salivary cortisol induces complete inhibition of latex attachment to the T or test line, a result that is indicated by a single line of latex deposition. Two blue lines indicate a negative result[54]. The control lines develop within approximately 5 minutes and the test is completed after 10 minutes.

The competitive lateral flow assay is a semi-quantifiable test. Use of Forsite Reader technology allowed the line intensity and, therefore, level of latex deposition to be calculated using reflectance photometry. The presence of a standard control line is used as a reference against the T or test line intensity[54].

2.6. Measurement of stress

The Depression Anxiety Stress Scales (DASS) questionnaire is simple and easy to use and was, therefore, selected for the current study. The Depression Anxiety Stress Scales (DASS) questionnaire has been assessed in various studies and was found to be reliable, valid and consistent[31, 56, 57, 58, 59, 60]. Reliability is defined as the degree of congruence at which a characteristic is measured. Validity is defined as the extent to which an instrument measures what it is meant to measure[31]. Consistency is defined as the ability to consistently measure a particular variable.

The Depression Anxiety Stress Scale (DASS) was developed by Lovibond and colleagues[56, 58]. Analysis of the Depression Anxiety Stress Scale 42 questionnaire (DASS – 42) has consistently presented a three factor structure as the optimal solution. The items of the depression scale focus on low mood, low self – esteem and poor outlook for the future. The anxiety scale items focus on a fear response and physiological arousal while the stress subscale focuses on persistent arousal and tension[58].

No studies have investigated the relationship between caries susceptibility, saliva buffering capacity and flow rates, stress and salivary cortisol levels.

Chapter 3

3. The present study

Early diagnosis of dental caries is essential to prevent progression of dental caries to endodontic involvement and loss of teeth. An untreated subclinical lesion forms a cavity that cannot be remineralized and requires a filling[2]. The caries status of patients can be successfully assessed using caries activity tests which include measurement of the salivary flow rate, buffering capacity of plaque and a diet analysis while predictors of caries activity include *Streptococcus mutans* and *Lactobacillus* counts in saliva[26]. Saliva flow rate is also important because it influences plaque pH and the composition of the plaque microflora[2, 3]. The buffering capacity of saliva is another important factor that controls the pH of the mouth[6].

The onset of caries has been found in individuals encountering stressful situations[61, 62, 63]. Salivary cortisol has frequently been used as a biomarker of stress[32, 47, 51]. Validation of Forsite's competitive lateral flow assay has been carried out on pigs and *ad hoc* studies have been conducted on human saliva samples and the tests are able to detect concentrations of salivary cortisol at levels of a few parts per million. The cortisol tests are simple to use, designed to work with fresh saliva samples allowing salivary cortisol levels to be determined at the chair side, no pre – treatment of the saliva is required.

3.1. Aims and Objectives

The aim of the study was to determine whether there is a correlation between active dental caries, the resting and stimulated flow rate of saliva, saliva buffering capacity, saliva cortisol levels and stress in patients attending a general dental practice in Lenasia South.

Chapter 4

4. Methods and Materials

4.1. Selection of patients

4.1.1. Experimental group

Thirty patients with active caries between the ages of 18 and 60 were selected for the experimental group. Exclusion criteria included the presence of Sjögren's Syndrome, any connective tissue diseases including Parkinson's and Alzheimer's disease, the use of drugs that may cause xerostomia and a history of current or previous irradiation. At the initial and subsequent visits teeth with active caries requiring treatment were restored with either amalgam or composite restorations, endodontic therapy or extractions.

One month after completion of the restorative dental treatment the patients were recalled for a one month follow up visit. The resting and stimulated salivary flow rates, buffering capacity of saliva and salivary cortisol levels were measured. In addition, the stress levels were measured again by requesting the patient to complete the DASS questionnaire (DASS – 42).

4.1.2. Control group

The control group consisted of 30 patients of the same age as the experimental group with either 32 teeth or a minimum of 24 teeth if they had undergone previous orthodontic treatment. Additional criteria were no active carious lesions, a Decayed Missing Filled Surfaces score (DMFS) of 4 or less and no orthodontic bands, removable orthodontic appliances, removable dentures, crowns, bridges or implants. Exclusion criteria were less than 24 teeth, patients with Sjögren's Syndrome, any connective tissue diseases including Parkinson's and Alzheimer's disease, the use of drugs that may cause xerostomia, or a history of previous or current irradiation.

The research project was explained before subjects were included in the study. Thereafter they were asked to sign a written informed consent form. The Human Research Ethics Committee of the Faculty of Health Sciences, University of the

Witwatersrand, Johannesburg granted ethical clearance (Ethical clearance no: M10246) before the study commenced.

4.2. Clinical caries examination

The examination was carried out by one qualified dentist. Patients were examined in the supine position in a dental chair provided with a halogen light. The teeth were dried with air and examined visually using a mirror and sharp probe, the visual – tactile criteria of Radlike and the methods described by Kidd and Joyston – Bechal were used[12]. The number of sound, decayed, missing and restored tooth surfaces was recorded.

4.3. Salivary flow rates

The resting and stimulated salivary flow rates were measured while the patient was seated comfortably in an upright position. A resting saliva sample was taken while the patient sat quietly and expectorated into a sputum jar for 10 minutes. A stimulated saliva sample was obtained by placing a drop of 2% citric acid on the centre of the tongue once every minute for 10 minutes while collecting saliva. The flow rate of the saliva samples was expressed as ml per minute.

4.4. Buffering capacity of saliva

The buffering capacity of the resting saliva was determined using a modified Dreizen test[14]. One drop of the pH indicators bromocresol green and bromocresol purple were added to 2ml of saliva. A 0.01N solution of lactic acid was titrated against the saliva until the colour changed from blue to green, indicating that the pH of saliva had changed from the resting pH to a pH of 4. The volume of 0.01N lactic acid required to change the pH was measured and the results were expressed as millilitres 0.01N lactic acid.

4.5. Salivary cortisol level

Forsite Diagnostics tested the reliability of the lateral flow assays by creating a standard curve using 75µl of 0 ng/ml, 2 ng/ml, 5 ng/ml, 10 ng/ml, 20 ng/ml, 50 ng/ml and 100 ng/ml concentrations of hydrocortisone – 21 – hemisuccinate sodium salt. In addition to ensure that the tests were reliable a standard curve was provided with each batch of lateral flow assays. The manufacturers also provided a standard

solution of cortisol containing 1ug/ml i.e.1000ng.ml which was diluted in Tris buffered saline + Triton X - 100 to give cortisol concentrations of 100ng/ml, 80ng/ml, 60ng/ml, 40ng/ml and 20ng/ml. 75µl of each of these concentration was tested using the lateral flow assays and read on the Forsite Reader to ensure that the reader detected increasing concentrations of cortisol accurately. In addition a reference device provided by Forsite Diagnostics was used to ensure that the Forsite Reader was configured correctly.

Resting saliva samples were collected from the patient and tested for salivary cortisol. The saliva samples were diluted in Tris buffered saline + Triton X – 100 which was used as a surfactant to aid the flow of the sample flow across the membrane. An aliquot of 75µl of the diluted buffered saliva sample was applied to the sample well of the test strip. After 10mins the test strips were placed in the Forsite Reader. The values appeared on the screen within 12 seconds. Data from the Forsite Reader was downloaded to a laptop for analysis. The test was repeated and an average of the two tests was used for statistical analysis.

The Forsite Reader measured the intensity of the T– line. The higher the salivary cortisol concentration the lighter the T– line value and the lower the value on the Forsite Reader. At low cortisol concentrations the T – line becomes dark and the Forsite Reader gives a high reading because sensitive latex particles which do not bind to salivary cortisol will attach to the T – line. With high concentrations of salivary cortisol fewer sensitive latex particles will remain unbound and bind to the T – line instead. The result is a low intensity T – line. However with low concentrations of salivary cortisol more sensitive latex particles will remain unbound to saliva and bind instead to the T – line. The result is the formation of a high intensity T – line.

Forsite Diagnostics provided a standard curve of the relationship between the T – line intensity and salivary cortisol concentration in ng/ml. This curve was used to determine the corresponding salivary cortisol concentration of each T – line intensity obtained using the Forsite Reader. The T – line intensity values were plotted against this standard curve and the corresponding salivary cortisol concentrations were determined.

4.6. Measurement of Stress using DASS – 42

The long version of the DASS (DASS – 42) consisting of three fourteen – question scales that measure depression, anxiety and stress was used in the study. Participants were asked to read each statement carefully and to indicate how each applied to them over the past week. The response categories for each scale ranged from 0 to 3 (0 = did not apply to me at all; 1 = applied to me to some degree, or some of the time; 2 = applied to me to a considerable degree, or a good part of time; and 3 = applied to me very much, or most of the time). Responses to statements pertaining to stress were combined to produce a total score, based on whether the participants were graded as normal, mildly stressed, moderately stressed, severely stressed or extremely severely stressed.

4.7. Statistical Analysis

The statistical analysis involved computing the descriptive statistics of the means and standard deviations of the DMFS scores, resting and stimulated salivary flow rates, buffering capacity and cortisol levels of saliva and the stress levels of the controls and experimental group. A chi–squared test was used to investigate the associations between the individual measurements, i.e. between age, gender, race and the mean DMFS scores, resting and stimulated flow rates, buffering capacity and cortisol levels of saliva and the stress levels of the controls and experimental group. The control and experimental groups were compared using the two sample t–test. The mean DMFS scores, resting and stimulated salivary flow rates, buffering capacity of saliva, salivary cortisol levels and stress levels were computed and presented by age, gender and race using the two sample t–test. The DMFS scores, resting and stimulated flow rates, buffering capacity and cortisol levels of saliva and stress levels of the controls and the experimental group at the initial visit and the after their teeth were restored were compared using the two sample t–test. The two sample t–test was used to assess significance at the 5% significance level, implying that if $p < 0.05$, then the differences between the two groups being compared are statistically significant. A multiple linear regression analysis was performed to investigate factors that were associated with our outcomes of interest, i.e. caries, salivary cortisol levels and stress levels.

Chapter 5

5. Results

Initially there were 60 participants in the study i.e. 30 controls and 30 experimental subjects. Four of the patients in the experimental group did not return for their second visit and the number of participants was reduced to 26 because they did not wish to continue with the study. The control group consisted of 14 females and 16 males whereas the experimental group was comprised of 14 females and 12 males. In total 14 Africans, 40 Indians, 1 Coloured and 1 Caucasian patient participated in the study. The overall mean age of the participants was 36.63 ± 12.23, the control group 32.33 ± 11.73 and the experimental group 42.12 ± 11.51.

5.1. Decayed Missing and Filled Surfaces index (DMFS)

The DMFS score was not dependent on age, gender or race. There was a statistically significant difference between the DMFS score of the control (0.40 ± 0.97) and experimental groups (29.27 ± 21.94) with $p < 0.001$. The mean number of tooth surfaces with active caries in the experimental group was 2.53. (Table 1)

5.2. Salivary flow rates

This study showed that the resting and stimulated salivary flow rates were not dependent on gender. However, the mean stimulated salivary flow rates (0.99 ± 0.56 ml/min) of the control group was dependent on age (32.33 ± 11.73) with $p = 0.048$. At the one month follow up visit the resting salivary flow rates of the Indians were significantly higher than the Africans and Coloureds, i.e. 0.89 ± 0.34 ml/min, 0.49 ± 0.23 ml/min and 0.45ml/min, respectively with $p = 0.014$. In addition, the mean resting and stimulated flow rates were higher in the control group than the experimental group i.e. 0.37 ± 0.30 ml/min and 0.99 ± 0.56 ml/min and 0.32 ± 0.19 ml/min and 0.84 ± 0.35 ml/min, respectively. These differences were not significant. There was a slight increase in the resting and stimulated salivary flow rates of the experimental group at the one month follow up visit, i.e. from

0.37 ± 0.16 ml/min to 0.88 ± 0.32 ml/min. However, this increase was not significant. (Table 1)

A comparison of the resting (0.37 ± 0.30 ml/min) and stimulated (0.99 ± 0.56 ml/min) salivary flow rates of the control group yielded a correlation of 0.571 which was significant at the 1% level. Results from the experimental group (0.32 ± 0.19 ml/min and 0.84 ± 0.35 ml/min) were similar with a correlation of 0.638 that was also significant at the 1% level. A comparison of the initial resting (0.32 ± 0.19 ml/min) and stimulated (0.84 ± 0.35 ml/min) salivary flow rates and buffering capacity (21.73 ± 9.77 ml 0.01N lactic acid) gave correlation values of 0.407 and 0.464 that were significant at the 5% and 1% level. Upon comparison of the resting (0.37 ± 0.16 ml/min) and stimulated (0.88 ± 0.32 ml/min) salivary flow rates and buffering capacity (22.25 ± 7.55 ml 0.01N lactic acid) at the one month follow up visit, the correlations were 0.662 and 0.444, respectively, with statistical significance at the 5% level. A comparison of the stimulated salivary flow rate (0.88 ± 0.32 ml/min) and buffering capacity (22.25 ± 7.55 ml 0.01N lactic acid) of the experimental group at the one month follow up visit gave a correlation of 0.381 which is statistically significant at the 5% level. (Table 2) Correlation values significant at the 1% and 5% levels imply that the odds are less than 1 out of 100 and 5 out of 100 that this is a chance occurrence. These correlation values suggest that there is a relationship between these variables.

5.3. Buffering capacity of saliva

The buffering capacity of saliva was not dependent on age, gender or race. There were no significant differences between the buffering capacity of saliva of the controls, 19.16 ± 8.99 ml 0.01N lactic acid, and experimental group, 21.73 ± 9.77 ml 0.01N lactic acid. At the one month follow up visit the buffering capacity of the experimental group increased from 21.73 ± 9.77 ml 0.01N lactic acid to 22.25 ± 7.55 ml 0.01N lactic acid but this difference was not statistically significant (Table 1).

5.4. Salivary cortisol

Salivary cortisol levels were not dependent on age, gender or race. There was no significant difference between salivary cortisol levels of the controls,

17.71 ± 22.51ng/ml, and the experimental group, 11.80 ± 16.61 ng/ml. There was a decrease in salivary cortisol levels at the one month follow up visit from 11.80 ± 14.61 ng/ml to 10.00 ± 12.12 ng/ml. However this difference was also not statistically significant (Table 1).

5.5. Depression Anxiety Stress Score (DASS)

The DASS score was not dependent on age, gender or race. There was no significant difference between the stress scores of the control, 11.33 ± 8.48, and the experimental groups, 11.20 ± 9.60, and the initial score of the experimental group, 11.20 ± 9.60, and the one month follow up visit with a score of 11.15 ± 9.79 (Table 1). Nineteen participants in the control group had normal stress levels, 4 were mildly, 4 moderately and 3 severely stressed. At the initial visit 24 subjects in the experimental group had normal stress levels, 2 were mildly stressed, 3 were severely stressed and 1 was extremely severely stressed. Four of the patients did not return for the examination at the one month follow up visit. The results of the remaining patients were similar, i.e. 22 of the 26 of the participants had normal stress levels. There was no change in the 3 severely stressed and the one extremely severely stressed subject (Figure 3).

A comparison of the DASS (11.20 ± 9.60) and the DMFS score (29.27 ± 21.94) and the presence of active caries in the experimental group yielded correlation values of 0.364 and 0.391, respectively. These were statistically significant at the 5% level. A comparison of the DASS score (11.15 ± 9.79) and DMFS score (29.27 ± 21.94) of the experimental group at the one month follow up visit found a correlation of 0.503 which is statistically significant at the 1% level (Table 2). This implies that the odds are less than 1 in a 100 that this is a chance occurrence.

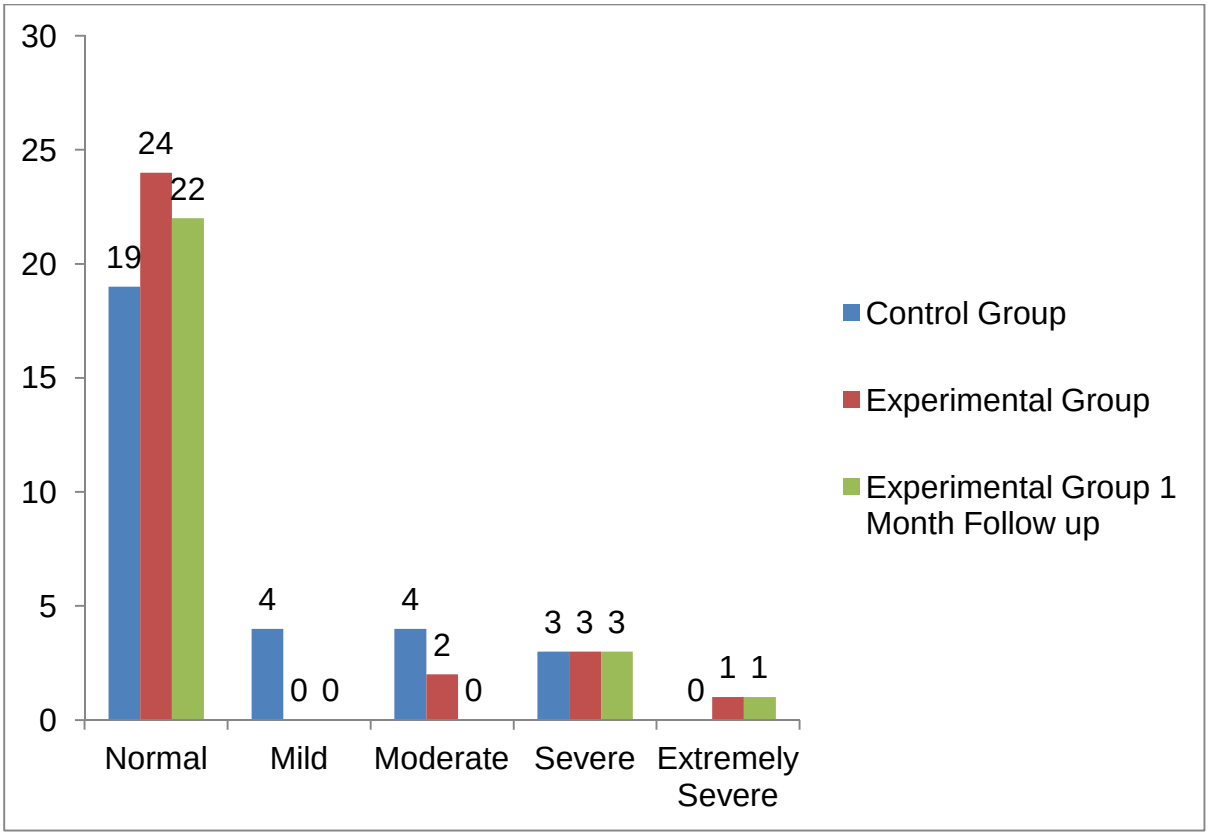


Figure 5.1 – The number of control and experimental subjects with normal, mild, moderate, severe and extremely severe stress levels at the initial visit and stress levels in the experimental subjects one month later.

Table 5.1 – The demographic characteristics and saliva flow, buffering capacity, salivary cortisol, Depression Anxiety Stress Scales score and DMFS of the control group, experimental group and experimental group at the 1 month follow up visit

Variable	Study Total	Control group	Experimental Group	Experimental Group(1 Month follow up)
Number of subjects	60	30	30	26
Demographic Characteristics				
		Mean ± S.D.		
Age	36.63 ± 12.23	32.33 ± 11.73	40.93 ± 11.32	42.12 ± 11.51
		Total		
Gender				
Male	30	16	14	12
Female	30	14	16	14
Race				
African	16	12	4	2
Caucasian	1	1	0	0
Coloured	1	0	1	1
Indian	42	17	25	23
Clinical Characteristics				
		Mean ± S.D.		
Saliva				
Flow (ml/min)				
Resting	0.34 ± 0.25	0.37 ± 0.30	0.32 ± 0.19	0.37 ± 0.16
Stimulated	0.92 ± 0.47	0.99 ± 0.56	0.84 ± 0.35	0.88 ± 0.32
Buffering capacity (ml 0.01N lactic acid)				
Resting	20.44 ± 9.40	19.16 ± 8.99	21.73 ± 9.77	22.25 ± 7.55
Salivary cortisol (ng/ml)	14.75 ± 19.05	17.71 ± 22.51	11.80 ± 14.61	10.00 ± 12.12
Dass score	11.27 ± 8.98	11.33 ± 8.48	11.20 ± 9.60	11.15 ± 9.79
DMFS	14.83 ± 21.19	0.40 ± 0.97*	29.27 ± 21.94*	N / A

*$p < 0.001$

Table 5.2 – Correlation matrices for comparison of different variables							
	Resting saliva flow	**Stimulated saliva flow**	**Buffering capacity**	**Salivary cortisol**	**DASS score**	**DMFS score**	**Active caries**
Control Group							
Resting saliva flow	1.000						
Stimulated saliva flow	0.571*	1.000					
Buffering capacity	0.112	0.101	1.000				
Salivary cortisol	-0.219	-.0271	-0.117	1.000			
DASS score	-0.227	-0.051	-0.281	0.079	1.000		
DMFS score	0.139	0.152	0.270	-0.044	-0.231	1.000	
Experimental Group							
Resting saliva flow	1.000						
Stimulated saliva flow	0.638*	1.000					
Buffering capacity	0.407#	0.464*	1.000				
Salivary cortisol	-0.121	-0.141	-0.156	1.000			
DASS score	0.287	0.036	0.310	-0.253	1.000		
DMFS score	-0.071	-0.051	-0.242	-0.082	0.364#	1.000	
Active caries	0.096	0.152	0.067	0.179	0.391#	0.368	1.000
Experimental Group (1 Month follow up visit)							
Resting saliva flow	1.000						
Stimulated saliva flow	0.662*	1.000					
Buffering capacity	0.444#	0.381#	1.000				
Salivary cortisol	-0.249	-0.113	-0.068	1.000			
DASS score	0.268	0.015	0.336	-0.230	1.000		
DMFS score	-0.086	-0.067	-0.134	-0.356	0.503*	1.000	

* = Statistically significant at 1% level (0.463); # = Statistically significant at 5% level (0.361)

Chapter 6

6. Discussion

6.1. Decayed Missing Filled Surfaces index (DMFS)

A patient's caries experience and future caries risk is indicated by their DMFS score[64]. The analysis showed there was a statistically significant difference between the DMFS scores of the control and experimental groups. This difference was due to the inclusion criterion for the control group who had a mean DMFS of 0.40 ± 0.97 which was significantly lower than the experimental group with a mean DMFS of 14.83 ± 21.19 and $p < 0.001$. These differences could be due to diet, oral hygiene and the variation in the degree of pathogenicity of the microorganisms. An increased caries experience is often found in patients following radiation therapy to the head and neck region, after use of medication which causes hyposalivation, in patients with substance abuse, psychogenic disorders and suffering from Sjögren's syndrome due to the reduced salivary flow rates[5, 11, 12]. However, the oral status of patients with reduced salivary flow rates can be improved with the use of artificial saliva replacements, chewing of xylitol chewing gum to stimulate saliva flow and by increasing the consumption of water.

6.2. Salivary flow rates

The results showed that resting and stimulated salivary flow rates were higher in the control than the experimental group. Numerous factors influence salivary flow rates including, the degree of hydration of subjects, body position, exposure to light, previous stimulation, circadian and annual rhythms, salivary gland size and drug use[65]. Salivary flow rates may also be influenced by oestrogen and thyroid hormone levels[64], which were not analysed in this study. A further study with a larger sample size may result in a more significant difference.

6.3. Buffering capacity of saliva

The saliva tests showed that the buffering capacity of the control group (19.16 ± 8.99 ml 0.01N lactic acid) was not significantly lower than the experimental group (21.73 ± 9.77 ml 0.01N lactic acid). However, there was an increase in the

buffering capacity of the experimental group from 21.73 ± 9.77 ml 0.01N lactic acid to 22.25 ± 7.55 ml 0.01N lactic acid at the one month follow up visit. These results differ from previous studies. The reason for these differences is not clear. However, the decrease in the ability of the control patients to buffer acids may be related to a change in the saliva because Jensdottir, Nauntofte, Buchwald and Bardow showed that whole mouth saliva composition may be changed by sucking acidic sweets[66]. The difference in saliva buffering capacity may also be related to stress as mentioned by Mentz[26]. The buffering capacity of saliva may also be exhausted by the ongoing production of organic acids by cariogenic bacteria in the mouth[67]. The rise in the buffering capacity of the experimental group at the one month follow up visit could be due to patient awareness. At the initial visit the dentist explained to patients what participation in the study entailed, how the research and measurements would be carried out and the reason for conducting these measurements. This may have provided the patients with information that made them aware of the importance of reducing the sugar content of their diet and could have caused an increase in the buffering capacity of saliva at their second visit. As a result the microorganisms would produce less acid from dietary carbohydrates and the buffering capacity of saliva would improve.

6.4. Salivary cortisol and Depression Anxiety Stress Scale score (DASS)

The DASS score of experimental group was 11.20 ± 9.60 which was similar to the control group i.e. 11.33 ± 8.48. However, the experimental group had lower salivary cortisol levels than the control group, i.e. 11.80 ± 14.61 ng/ml and 17.71 ± 22.51 ng/ml, respectively. This suggests that the experimental group may be less stressed than the controls. One of the reasons may be that the DASS questionnaire is subjective and is based on the past week of the subject's life because the questionnaire asked subjects to answer questions based on how much the statement "applied to them over the past week".

Other questionnaires that are less subjective may have confirmed the correlation between cortisol levels and stress. In addition other methods of stress measurement that could have been used include the Lipp's Stress Symptoms Inventory which is based on symptoms of stress and would, therefore, be less subjective, the Perceived Stress Questionnaire, the Perceived Stress Scale, the Index of Clinical Stress, the

Stress Response Inventory or the Trier Inventory for the Assessment of Chronic Stress. Furthermore laboratory methods of salivary cortisol measurement including the ELISA extraction – free technique[52], Dissociation – enhanced kanthaside fluoroimmunoassay (DELFIA) [53], nano – linker chemistry coupled with surface plasmon resonance detection[49], liquid chromatography – tandem mass spectrometry (LC – MS/MS)[50], enzyme immunoassay[34], flow – filtered portable surface plasmon resonance[48], automated – in tube – solid phase microextraction (SPME) coupled liquid chromotography – mass spectrometry (LC / MS)[51] may have found a better correlation between stress and salivary cortisol**.**

6.5. Limitations of the current study

There were several limitations of the study. A larger sample size may have resulted in a better indication of the correlation between the variables whereas a longer time interval between the initial consultation and the second visit and any subsequent visits may have yielded results that were more significant. A dietary analysis at the initial consultation and the follow up visit may have indicated whether the diet had influenced any of the variables. Furthermore if the study had been extended over a longer time period individuals with high stress levels could have been referred to a psychologist for treatment. This would have made it possible to determine whether treatment had any influence on the variables investigated.

Chapter 7

7. Conclusion

The results of this study suggest that patients with active caries have lower resting and stimulated salivary flow rates than caries free patients. This confirms there is a positive correlation between active dental caries and salivary flow rate as has been reported by other authors[5, 10, 11, 12, 13, 14]. Patients with active dental caries had a better buffering capacity of saliva than patients with no caries. This indicates a negative correlation between the two and is contradictory to the research of other authors[1, 19, 20, 22, 23, 25]. Patients with active dental caries also had lower salivary cortisol concentrations and stress level than patients with no caries. Our results differ from previous research which found that patients with active caries have higher salivary cortisol concentrations and stress levels than patients with no caries[4, 28, 43]. This suggests that salivary cortisol concentrations and stress levels are not related to caries.

This study has shown that the methods used to measure salivary flow rates, buffering capacity of saliva and the salivary cortisol concentration can be used at the chair side in the dental surgery to assess the caries susceptibility of an individual. However, a more accurate stress questionnaire should be used. Measuring salivary flow rates and the buffering capacity of saliva at the chair side will enable dentists to identify patients that are at a higher risk of developing caries so that preventative measures can be taken.

The present observations merit further studies on the long term effects of active caries on the buffering capacity of saliva, salivary cortisol levels and stress levels. Further studies may reveal a closer relationship between stress and caries.

Further studies on the effect of caries removal followed by the placement of a restoration on buffering capacity of saliva are required to determine whether there is a greater change in the buffering capacity of saliva of patients with many large carious lesions compared to patients with a few small lesions. In addition the

influence of diet on the buffering capacity of saliva needs to be investigated. Further studies are also required to investigate the relationship between stress levels and salivary cortisol levels in patients with extensive carious lesions and restorations including crowns, bridges and implants.

APPENDIX A

List of abbreviations used in the text and tables

et al	et alii, et aliae, et alia
DASS	Depression Anxiety Stress Scale
DASS – 42	Depression Anxiety Stress Scale – 42 questions
DMFS	Decayed Missing Filled Surfaces
ml/min	millilitres per minute
ng/ml	nano grams per millilitre
N	Normal
ml	millilitres
µl	microlitres
S.D.	Standard deviation

APPENDIX B

Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Priyesh G Hira

CLEARANCE CERTIFICATE	**M10246**
PROJECT	Is Stress Responsible for the Extensive Amount of Dental Caries seen in Patients with Full Mouth Prosthodontic Rehabilitation?
INVESTIGATORS	Dr Priyesh G Hira.
DEPARTMENT	School of Dentistry/Oral Microbiology
DATE CONSIDERED	26/02/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 30/04/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof M Coogan

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

Division of Oral Microbiology

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Department of Clinical Microbiology and Infectious Diseases
Faculty of Health Sciences
7 York Rd, Parktown, Johannesburg, 2193

Private Bag 3
WITS, 2050, South Africa
Tel: 27 11 646 5959
Fax: 27 865533018

Professor PE Cleaton Jones
Chairman Wits Human Research Ethics Committee
Faculty of Health Sciences
University of the Witwatersrand
Johannesburg

7th November 2012

Dear Professor Cleaton Jones,

Re: Request for Ethics Clearance Certificate – Priyesh Hira MSc Dent

Graduate Studies of the Faculty of Health Sciences acting on behalf of Senate has agreed to award Priyesh Hira the degree of Master of Dentistry for his research report entitled * **Buffering capacity of saliva, salivary flow rates and cortisol levels in patients with active dental caries**.

Ethics clearance was obtained on 30th April 2010 **M10246** for a study entitled **Is stress responsible for the extensive amount of dental caries seen in patients with full mouth prosthodontic rehabilitation?** At a subsequent meeting the ethics committee requested a change of title. Please could you provide Dr Hira with the new clearance certificate in order that he may graduate in December 2012.

Thank you for your kind attention

Yours sincerely

Prof Maeve Coogan
Supervisor

UNIVERSITY OF THE WITWATERSRAND
PROF PE CLEATON - JONES
HREC (MEDICAL)
2012 -11- 09
JOHANNESBURG

* This altered title has been approved by the HREC (Medical), the revised clearance appears to have been misplaced. Another will now be issued by Mrs Anisa Kishaw as soon as she returns from sick leave. PE Cleaton Jones 09/11/12
DR Hira may graduate

APPENCIX C

Forms for participants

Department of Oral Microbiology
School of Oral Health Sciences
University of the Witwatersrand

INFORMATION SHEET AND CONSENT FORM

Research: Buffering Capacity of Saliva, Salivary Flow Rates and Cortisol Levels in Patients With Active Caries

Good day patient,

I Dr. Priyesh Gunvant Hira, a postgraduate student in the School of Dentistry am undertaking a research project to study the influence of stress on your dental health. I wish to understand how you control the bacteria in your mouth that produce acids and cause decay. To do this I will examine your saliva and measure your stress level over a period of a week.

I am inviting you to take part in this research study. The study will examine 60 patients who attend my practice, 30 with no decay and 30 with decay

Participation in the study will entail an examination of your mouth and teeth during which I will record the number of decayed, missing and filled teeth in your mouth. In addition, I will need to collect 2 samples of saliva and ask you to fill in Depression Anxiety Stress Scales Questionnaire. You will be examined in my Dental Surgery and it will take approximately 30 minutes of your time.

The examination should not cause any discomfort or pain. The advantages are that you will be informed if there are any problems in your mouth and you will be made aware of the factors that may have contributed to tooth decay in your mouth. If you have any decay I will treat the tooth and ask you to return after 1 month to repeat the tests and to see if there has been an improvement.

This is a voluntary study and if you do not feel comfortable or happy you may withdraw at any time without any disadvantage to yourself. There will be no financial costs to you.

This information will be confidential because I will use a coding system to protect your identity and the results will be used for research purposes only. Effort will be made to keep personal information confidential. Organizations that may inspect and / or copy your research records for quality assurance and data analysis include groups such as the Research Ethics Committee.

For further information / reporting of study related adverse effects please contact:
Dr. P. G. Hira
Tel: (011) 854 – 1738
Cell: 084 – 657 – 5545

To report complaints or problems please contact the Research Ethics Committee
Tel: (011) 717 – 1234

For confidentiality reasons please quote your study number reflected below when communicating with the researcher.

Study number:

I thank you for taking the time to read this information sheet. If you are willing to participate please sign the relevant portion (consent of the participant).

Yours sincerely

_____
Dr. Priyesh Gunvant Hira

Please note: Please delete the option which does not apply.

I _____ hereby consent to participate in the study. I have read and have / have not understood the information sheet and do / do not understand what will be required of me.

Study number:

_____
Signature

_____
At

_____
Date

DASS

Study no: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found myself getting upset by quite trivial things	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I just couldn't seem to get going	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I had a feeling of shakiness (eg, legs going to give way)	0	1	2	3
8	I found it difficult to relax	0	1	2	3
9	I found myself in situations that made me so anxious I was most relieved when they ended	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting upset rather easily	0	1	2	3
12	I felt that I was using a lot of nervous energy	0	1	2	3
13	I felt sad and depressed	0	1	2	3
14	I found myself getting impatient when I was delayed in any way (eg, lifts, traffic lights, being kept waiting)	0	1	2	3
15	I had a feeling of faintness	0	1	2	3
16	I felt that I had lost interest in just about everything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I perspired noticeably (eg, hands sweaty) in the absence of high temperatures or physical exertion	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life wasn't worthwhile	0	1	2	3

Please turn the page ☞

Reminder of rating scale:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

22	I found it hard to wind down	0	1	2	3
23	I had difficulty in swallowing	0	1	2	3
24	I couldn't seem to get any enjoyment out of the things I did	0	1	2	3
25	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
26	I felt down-hearted and blue	0	1	2	3
27	I found that I was very irritable	0	1	2	3
28	I felt I was close to panic	0	1	2	3
29	I found it hard to calm down after something upset me	0	1	2	3
30	I feared that I would be "thrown" by some trivial but unfamiliar task	0	1	2	3
31	I was unable to become enthusiastic about anything	0	1	2	3
32	I found it difficult to tolerate interruptions to what I was doing	0	1	2	3
33	I was in a state of nervous tension	0	1	2	3
34	I felt I was pretty worthless	0	1	2	3
35	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
36	I felt terrified	0	1	2	3
37	I could see nothing in the future to be hopeful about	0	1	2	3
38	I felt that life was meaningless	0	1	2	3
39	I found myself getting agitated	0	1	2	3
40	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
41	I experienced trembling (eg, in the hands)	0	1	2	3
42	I found it difficult to work up the initiative to do things	0	1	2	3

APPENDIX D

Analyses recording forms

Study no:_____

Date of examination:_____

DMFS:_____

Active caries (If applicable):_____

Resting saliva flow rate:_____ml per 10 minutes

Stimulated saliva flow rate:_____ml per 10 minutes

Buffering capacity of saliva:_____ml 0.01N lactic acid

Salivary cortisol 1:_____

Salivary cortisol 2:_____

Salivary cortisol average:_____

DASS:_____

Stress level:_____

1 Month follow up visit (If applicable):

Date of examination:_____

DMFS:_____

Resting saliva flow rate:_____ml per 10 minutes

Stimulated saliva flow rate:_____ml per 10 minutes

Buffering capacity of saliva:_____ml 0.01N lactic acid

Salivary cortisol 1:_____

Salivary cortisol 2:_____

Salivary cortisol average:_____

DASS:_____

Stress level:_____

Chapter 8

8. List of references

1. Preethi, B. P, Pyati, A, Dodawad, R. Evaluation of the Flow Rate, pH, Buffering Capacity, Calcium, Total Protein and Total Antioxidant Levels of Saliva in Caries Free and Caries Active Children – An in vivo Study. Biomedical Research 2010;21(3):289 – 294.
2. Kidd, E, Joyston-Bechal, S. Essentials of dental caries: The disease and its management. Bristol. IOP Publishing Limited, 1987, Chapter 1. pp. 1 – 15.
3. Krasse, B. Caries Risk: A Practical Guide for Assessment and Control. Chicago. Quintessence Publishing Co, 1985, Chapter 1. pp. 15 – 28.
4. Rai, K, Hegde, A. M, Shetty, S, Shetty, S. Estimation of Salivary Cortisol in Children with Rampant Caries. Journal of Clinical Paediatric Dentistry 2010;34(3):249 – 252.
5. Edgar, W. M, Highman, S. M, Manning, R. H. Saliva Stimulation and Caries Prevention. Advances in Dental Research 1994;8(2):239 – 245.
6. Malekipour, M. R, Messripour, M, Shirani, F. Buffering Capacity of Saliva in Patients with Active Dental Caries. Asian Journal of Biochemistry 2008;3(5):280 – 283.
7. Crawford, J. R, Henry, J. D. The Depression Anxiety Stress Scales: Normative Data and Latent Structure in a Large Non – Clinical Sample. British Journal of Clinical Psychology 2003;42:111 – 131.
8. Deal With Stress (homepage on the Internet). Available from: http://www.dealwithstress.com/What-Is-Psychological-Stress-.html.
9. Edgar, W. M. Sugar Substitutes, Chewing Gum and Dental Caries – A Review. British Dental Journal 1998;184(1):29 – 32.
10. Lagerlöf, F, Oliveby, A. Caries – protective Factors in Saliva. Advances in Dental Research 1994;8(2):229 – 238.
11. Akpata, E. S, Al – Attar, A, Sharma, P. N. Factors Associated with Severe Caries among Adults in Kuwait. Medical Principles and Practice 2009;18(2):93 – 99.

12. Flink, H. Studies on the Prevalence of Reduced Salivary Flow Rate in Relation to General Health and Dental Caries, and Effect of Iron Supplementation [thesis]. Karolinska Institutet, 2007.
13. Stennson, M, Wendt, L. K, Koch, G, Oldaeus, G, Lingström, P, Birkhed, D. Caries Prevalence, Caries – Related Factors and Plaque pH in Adolescents with Long – Term Asthma. Caries Research 2010;44(6):540 – 546.
14. Coogan, M. M, Mackeown, J. M, Galpin, J. S, Fatti, L. P. Microbiological Impressions of Teeth, Saliva and Dietary Fibre can Predict Caries Activity. Journal of Dentistry 2008;36(11):892 – 899.
15. Stookey, G. K. The Effect of Saliva on Dental Caries. Journal of American Dental Association 2008;139(Supp):11S – 17S.
16. Moritsuka, M, Kitasako, Y, Ikeda, M, Nomura, S, Tagami, J. Relationship among Salivary Acid Buffering Capacity, DMFT, and Flow Rate. Abstract number 2079, 81st session of IADR 25 – 28 June 2003.
17. de Castilho, H. R, Pardi, V, Pereira, C. V. Dental Caries Experience in Relation to Salivary Findings and Molecular Identification of S. mutans and S. sobrinus in Subjects with Down Syndrome. Odontology 2011;99(2):162 – 167.
18. Watanabe, Y, Mizoguchi, H, Masamura, K, Nagaya, T. No Relationship of Salivary Flow Rate and Secretory Immunoglobulin A to Dental Caries in Children. Environmental Health and Preventive Medicine 1997;2(3):122 – 125.
19. Mass, E, Gadoth, N, Harell, D, Wolff, A. Can Salivary Composition and High Flow Rate Explain the Low Caries Rate in Children with Familial Dysautonomia? Paediatric Dentistry 2002;24(6):581 – 586.
20. Kusaik, A, Kochanska, B, Limon, J, Zoltowska, A, Zedler, E, Swietlik, D, Kowalska – Skabara, J. Buffering Capacity and Caries Prevalence in Turner's Syndrome. Dental Forum 2010;2:21 – 25.
21. Jeong, S. – J, Apostolska, S, Jankulovska, M, Angelova, D, Nares, S, Yoon, M. – S, Lim, D. – S, Angelov, N, Jeong, M. – J. Dental Caries Risk can be Predicted by Simply Measuring the pH and Buffering Capacity of Saliva. Journal of Dental Hygiene Science 2006;6(3):159 – 162.
22. Agus, H. M, Schamschula, R. G. Lithium Content, Buffering Capacity and Flow Rate of Saliva and Caries Experience in Australian Children. Caries Research 1983;17(2):139 – 144.

23. Coogan, M. M, Motlekar, H. B. Salivary and plaque acids in caries active and caries free subjects. Journal of the Dental Association of South Africa 1996;51(12):823 – 827.
24. Kinirons, M. J. Increased Salivary Buffering in Association with a Low Caries Experience in Children Suffering from Cystic Fibrosis. Journal of Dental Research 1983;62(7):815 – 817.
25. Davis, P. B. Pathophysiology of Cystic Fibrosis with Emphasis on Salivary Gland Involvement. Journal of Dental Research 1987;66:667 – 671.
26. Mentz, N. J. An alginate impression method to detect dental caries in patients with crown and bridge restorations [thesis]. (M Dent, University of the Witwatersrand, Johannesburg), 2007.
27. Meurman, J. H, Rantonen, P. Salivary Flow Rate, Buffering Capacity, and Yeast Counts in 187 Consecutive Adult Patients from Kuopio, Finland. Scandinavian Journal of Dental Research 1994;102(4):229 – 234.
28. Wikner, S, Söder, P. O. Factors Associated with Salivary Buffering Capacity in Young Adults in Stockholm, Sweeden. Scandanavian Journal of Dental Research 1994;102(1):50 – 53.
29. Boyce, W. T, Den Besten, P. K, Stamperdahl, J, Zhan, L, Jiang, Y, Adler, N. E, Featherstone, J. D. Social Inequalities in Childhood Dental Caries: The Convergent Roles of Stress, Bacteria and Disadvantage. Social Science and Medicine 2010;71(9):1644 – 1652.
30. Shigeyama, C, Ansai, T, Awano, S, Soh, I, Yoshida, A, Hamasaki, T, Kakinoki, Y, Tominaga, K, Takahashi, T, Takehara, T. Salivary Levels of Cortisol and Chrmomgranin A in Patients with Dry Mouth Compared with Age – Matched Controls. Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontic 2008;106(6):833 – 899.
31. Apóstolo, J. L, Mendes, A. C, Azeredo, Z. A. Adaptation to Portuguese of the Depression, Anxiety and Stress Scales (DASS). Rev Latino - Am Enfermagem 2006;14(6):863 – 871.
32. Garcia, M. C, Souza, A, Bella, G. P, Grassi – Kassisse, . M, Tacla, A. P, Spadari – Bratfisch, R. C. Salivary Cortisol Levels in Brazilian Citizens of Distinct Socioeconomic and Cultural Levels. Stress, Neurotransmitters and Hormones 2008;1148:504 – 508.

33. Laudenslager, M. L, Noonan, C, Jacobsen, C, Goldberg, J, Buchwald, D, Bremner, J. D, Vaccarino, V, Manson, S. M. Salivary cortisol among American Indians with an without posttraumatic stress disorder (PTSD): Gender and alcohol influences. Brain Behaviour and Immunity 2009:1 – 5.
34. Da Silva Andrade, A, Gamero, G. H, Pereira, L. J, Junqueira Zanin, I. C, Gaviao, M. B. Salivary cortisol levels in young adults with temporomanibular disorders. Minerva Stomatologica 2008;57(3):109 – 116.
35. Haussmann, M. F, Vleck, C. M, Farrar, E. S. A laboratory exercise to illustrate increased salivary cortisol in response to three stressful conditions using competitive ELISA, Advances in Physiology Education 2007;31(1):110 – 115.
36. Obel, C, Hedegaard, M, Henriksen, T. B, Secher, N. J, Olsen, J, Levine, S. Stress and salivary cortisol during pregnancy. Psychoneuroendocrinology 2005;30(7):647 – 656.
37. Weekes, N. Y, Lewis, R. S, Goto, S. G, Garrison – jakel, J, Pate, F, Lupien, S. The effect of an environmental stressor on gender differences on the awakening cortisol response. Psychoneuroendocrinology 2008;33(6):766 – 772.
38. Ng, V, Koh, D, Chai, S. – E. Examination stress, salivary cortisol, and academic performance. Psychological Reports 2003;93(3):1133 – 1134.
39. Ng, V, Koh, D, Mok, B. Y. Y, Chia, S. – E, Lim, L. – P. Salivary Biomarkers Associated with Academic Assessment Stress Among Dental Undergraduates. Journal of Dental Education 2003;67(10):1091 – 1094.
40. Maina, G, Palmas, A, Filon, F. L. Relationship between self – reported mental stressors at the workplace and salivary cortisol. International Archives of Occupational and Environmental Health 2008;81(4):391 – 400.
41. Dahlgren, A. Different levels of work – related stress and the effects on sleep, fatigue and cortisol. Scandinavian Journal of Work, Environment and Health 2005;31(4):277 – 285.
42. Bakke, M, Tuxen, A, Thomsen, C. E, Bardow, A, Alkjaer, T, Jensen, B. R. Salivary Cortisol Level, Salivary Flow Rate, and Masticatory Muscle Activity in Response to Acute Mental Stress: A Comparison between Aged and Young Women. Gerontology 2004;50(6):383 – 392.
43. Takai, N, Yamaguchi, M, Aragaki, T, Eto, K, Uchihashi, K, Nishikawa, Y. Effect of psychological stress on the salivary cortisol an amylase levels in healthy young adults. Archives of Oral Biology 2004;49(12):963 – 968.

44. Akyuz, S, Pince, S, Hekin, N. Children's stress during a restorative dental treatment: Assessment using salivary cortisol measurements. Journal of Clinical Pediatric Dentistry 1996;20(3):219 – 223.
45. Kugler, J, Reintjes, F, Tewes, V, Scheslowski, M. Competition stress in soccer coaches increases salivary Immunoglobin A and salivary cortisol. The Journal of Sports Medicine and Physical Fitness 1996;36(2):117 – 120.
46. Tarui, H, Nakamura, A. Saliva cortisol: A good indicator of acceleration stress. Aviation, Space, and Environmental Medicine 1897;58(6):573 – 575.
47. Hellhammer, D. H, Wust, S, Kudielka, B. M. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 2009;34(2):163 – 171.
48. Stevens, R. C, Soelberg, S. D, Near, S, Furlong, C. E. Detection of Cortisol in Saliva with a Flow – Filtered, Portable Surface Plasmon Resonance Biosensor System. Analytical Chemistry 2008;80(17):6747 – 6751.
49. Mitchell, J. S, Lowe, T. E, Ingram, J. R. Rapid ultrasensitive measurement of salivary cortisol using nano – linker chemistry coupled with surface plasmon resonance detection. The Analyst 2009;134(2):380 – 386.
50. Perogamvros, I, Owen, L. J, Keevil, B. G, Brabant, G, Trainer, P. J. Measurement of salivary cortisol with liquid chromatography – tandem mass spectrometry in patients undergoing dynamic endocrine testing. Clinical Endocrinology 2009:1 – 16.
51. Kataoka, H, Matsuura, E, Mitani, K. Determination of cortisol in human saliva by automated in – tube solid – phase microextraction coupled with liquid chromatography – mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 2007;44(1):160 – 165.
52. Greabu, M, Purice, M, Totan, A, Spinu, T, Totan, C. Salivary cortisol – marker of stress response to different dental treatment. Romanian Journal of Internal Medicine 2006;44(1):49 – 59.
53. Hoferl, M, Krist, S, Buchbauer, G. Adaptation of DELFIA^{TM} Cortisol Kit for Determination of Salivary Cortisol Concentration. Archiv der Pharmazie. Chem. Life. Sci 2005;338(10):493 – 497.
54. Forsite Diagnostics Limited, August 2009, Sand Hutton, York, YO41 1LZ, UK. www.forsitediagnostics.com
55. Fliege, H, Rose, M, Arck, P, Walter, O. B, Kocalevent, R. – D, Weber, C, Klapp, B. F. The Perceived Stress Questionnaire (PSQ) Reconsidered: Validation and

Reference Values From Different Clinical and Healthy Adult Sample. Psychosomatic Medicine 2005;67:78 – 88.

56. Henry, J. D, Crawford, J. R. The Short – Form Version of the Depression Anxiety Stress Scales (DASS – 21): Construct Validity and Normative Data in a Large Non – Clinical Sample. British Journal of Clinical Psychology 2005;44:227 – 239.
57. Shea, T. L, Tennant, A, Pallant, J. F. Rasch Model Analysis of the Depression, Anxiety and Stress Scales (DASS). Bio Med Central Psychiatry 2009;9(21).
58. Depression Test (homepage on internet). Available from: http://www.depression-test.net/depression-anxiety-and-stress-scale.html
59. Ownsworth, T, Little, T, Turner, B, Hawkes, A, Shum, D. Assessing Emotional Status Following Acquired Brain Injury: The Clinical Potential of the Depression, Anxiety and Stress Scales. 2008;22(11):858 – 869.
60. Suman, M, Spalj, S, Plancak, D, Dukic, W, Juric, H. The influence of war on the oral health of professional soldiers. International Dental Journal 2008;58(2):71 – 74.
61. Honkala, E, Maidi, D, Kolmakow, S. Dental caries and stress among South African political refugees. Quintessence International 1992;23(8):579 – 583.
62. Sutton, P. R. N. Psychosomatic Dental Disease: Is Mental Stress in Adults Followed by Acute Dental Caries in all Racial Groups? Medical Hypotheses 1993;41(3):279 – 281.
63. Bronkhorst, A. Effect of probiotic chewing gum on mutans streptococci and lactobacilli in orthodontic patients [Thesis]. (MDent, University of the Witwatersrand, Johannesburg), 2011
64. Dawes, C. Salivary flow patterns and the health of hard and soft oral tissues. Journal of the American Dental Association 2008;139:18 – 24.
65. Jensdottir, T, Nauntofte, B, Buchwald, C, Bardow, A. Effects of sucking acidic candy on whole-mouth saliva composition. Caries Research 2005;39:468 – 474.
66. Moritsuka, M, Kitasako, Y, Burrow, M. F, Ikeda, M, Tagami J. The pH change after HCl titration into resting and stimulated saliva for a buffering capacity test. Australian Dental Journal 2006;51:170 – 174.