STRESS AND SALIVARY BIOMARKERS AMONG
DENTAL UNDERGRADUATES
- A LONGITUDINAL STUDY

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I am also blessed for having my family members and personal friends who have being

constantly given me their unconditional support.

Finally, I want to thank God for being the One who provides me with everything.

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SUMMARY

Objectives: To a) investigate perceived sources and levels of stress experienced by dental undergraduates throughout the academic year (AY) (b) study the association of personality, coping strategies and social support on stress levels and (c) investigate the relationship between salivary immunoglobulin A (IgA), lysozyme and cortisol with self-perceived stress.

Methods: All NUS dental undergraduates (n=134) were invited to participate in this study. Participants completed a self-administered questionnaire and contributed a salivary sample at the beginning (T1), middle (T2) and the end (T3) of the AY. The questionnaire collected sociodemographic data, perceived stress measured on the Dental Environmental Stress (DES) questionnaire, General Life Events (GLE) scale, Perceived Stress Scale (PSS), mental health questionnaire (GHQ-28); and information on Type-A behaviour, coping strategies and social support. The DES had questions on stress from academic work (AW), clinical factors (CF), faculty/administration factors (FF) and personal factors (PF). Enzyme-linked immunosorbent assay was used to determine salivary cortisol, IgA and lysozyme levels.

Results: The questionnaire response rates (>98%) and valid subjects for saliva analyses (71%) were satisfactory. The top ranked DES subscale was AW throughout the AY (mean scores between 2.79 and 2.89). A small proportion of participants had GLE scores of >50 points. Overall, dental undergraduates had higher PSS at T2. Females had higher scores than males on PSS at T1 (21.37 vs 18.98, p=0.02) and T2 (22.24 vs 19.92, p=0.02). At T2, 4th year participants had higher PSS score than the 1st year students (23.55 vs 18.97, p=0.02). The proportions of participants who scored GHQ $\geq$ 5 were 46.3% (T1), 56.4% (T2) and 63.6% (T3). There were statistically significant positive correlations between DES, GLE and PSS with GHQ. The correlation coefficients ranged from 0.26 to 0.76.

75.4% of participants possess a Type-A personality. Females had higher scores on less-useful coping strategies than males. There was no significant difference in various coping strategies adopted by participants from different academic classes. Participants indicated that they could rely on their family members/friend (T1=85.1%, T3=77.3%) and course-mates (T1=8.2%, T3=75.0%) when they were in need.

Type-A personality was positively associated with GHQ (r=0.20, p=0.02) and PSS (r=0.15, p=0.07). Problem-focused coping was negatively correlated with GHQ (r=-0.18, p=0.04) at T3 and PSS (r_s =-0.25, -0.20; p_s <0.05) at T1 and T3, respectively. Social support was negatively associated with PSS and GHQ.

Females had significantly higher IgA secretion rates that males at T1 ([GM]: 48.37 vs 62.75 μg/min) and T2 (GM: 53.63 vs 74.78 μg/min). There was no significant difference between males and females for the other biomarkers at different times of the AY. Significant correlations between stressors and perceived stress level were only noted at T2. At T2, PSS was positively correlated with cortisol concentration (r=0.33, p=0.00) and inversely associated with IgA (r=-0.20, p=0.05) and lysozyme (r=-0.18, p=0.06).

Conclusion:

Academic work was the prime stressor for participants regardless of academic class or gender. They perceived high levels of stress during the middle of the AY. Most participants had Type-A behaviour. They usually adopted problem-focused coping and sought support from family members/friends and course-mates. There was a positive relationship between self-perceived stress levels with cortisol and inverse relationships with IgA and lysozyme.

Key Words: dental environmental stress, moderating factors of stress, saliva, IgA, lysozyme, cortisol

CHAPTER 1

INTRODUCTION

Tertiary education is regarded as a transition period to prepare the individual to move to a future profession. It has always been recognised as highly stressful (Ko et al., 1999; Mosley et al., 1994). Academic pressure and demands for academic excellence are the primary sources of stress in undergraduates. Besides, undergraduates may face the stresses arising from the lack of immediate support from their family, experience the making and breaking relationships and endure anxieties about the general state of the world (Heath et al., 1999; Ko et al., 1999).

While certain degrees of stress are required to stimulate the students, increasing stress excessively may result in declining students performance (Westerman et al., 1993).

It has been reported that the dental profession was ranked the most stressful when compared to other health care professions (Cooper CL, 1987). Many studies have been conducted to address the stress of dental students. Due to the differences in social-cultural background, and personal beliefs and attitudes, students in different countries perceive stressors in their dental environments differently. Moreover, those studies were cross-sectional in nature and used descriptive and subjective methods to assess students' stress. So far there was only one cross-sectional study conducted in Singapore to compare the differences in students' perception of dental school stress between Singapore and an American cohorts (Yap et al., 1996) The study was conducted about a decade ago. During the past decade, the dental curriculum in

Singapore has been changed substantially from the traditional discipline-based system to the current competency-based curriculum and problem-based learning methods as well as in social changes.

Currently in Singapore, the course for the Bachelor of Dental Surgery is a 4-year program and is only available in the National University of Singapore. The faculty enrolls 34 new students a year. During the 4-year program, students are exposed to both the academic and practical elements of dentistry. The first two years of study cover basic sciences and preclinical training. In the third and final years, the management and treatment of patients are strongly emphasized along with teaching of oral and dental diseases. (Information obtained from: Faculty of Dentistry, undergraduate entry requirements).

The dental course in Singapore can be considered intensive, and thus would impose a high degree of stress to the students.

It was noted that psychological distress in first-year medical undergraduates was predictive of occupational stress in later years (Guthrie et al., 1998). Heath et al also reported that stressful events experienced during undergraduate training may have some influence on the future profession (Heath et al., 1999). Identification and clearer understanding of potential stressors in the dental environment in the early years of training and their impact on students will help students and faculty to find appropriate approaches in handling students' stress (Humphris et al., 2002; Westerman et al., 1993).

In recent years, there is an increased interest in using biomarkers as a more objective way to measure stress and to complement questionnaire based stress assessments.

In order to obtain a comprehensive understanding of dental environment stress in Singapore, we carried out this longitudinal study to investigate the stress patterns of dental undergraduates throughout the academic year as well as to study the moderating effect of personality, coping strategies and social support on their stress levels. In addition, this study also investigates the relationship of salivary biomarkers with the students' self-perceived stress levels.

This study, being the first longitudinal study to assess the stress of dental undergraduates in Singapore will differ from other studies in a few areas:

1. It will help us to understand the perceived stress and stressors patterns of dental undergraduates in Singapore throughout the academic year.
2. It will investigate the moderating effect of personality, social support and coping strategies on students' stress levels.
3. It will be the first study to use salivary biomarkers as a more objective way to complement the subjective assessment of dental undergraduates' stress.

CHAPTER 2

LITERATURE REVIEW

2.1   Stress

2.1.1   Definition / concept of stress

Stress is a word derived from the Latin word *stringere,* meaning "to draw tight", and was used to describe hardships or affliction. Subsequently it was used to denote 'force, pressure, strain or strong effort' placed upon an individual's organ or mental power (Hinkle, 1974). Stress is a prevailing issue in this modern society. It affects all of us regardless our gender, age, race or class.

Nevertheless, the definition of stress still remains vague. It had been variously defined in terms of a stimulus by Cannon in 1932, a non-specific physiological response by Selye in 1955 (Blonna, 1996) and more recently, in terms of the reaction to situation following cognitive appraisal by Lazarus and Folkman (Lazarus and Folkman, 1984).

In short, stress is a response made by people to demands made upon them. The agent that triggers a stress response within the body is called a stressor. The stressor is the cause and stress is the effect. What causes the stress may be immaterial, and the degree of readjustments one's has to adapt to the stressor will determine the consequences (Bailey, 1987).

2.1.2 Process of stress

Tom Cummings and Cary Cooper have designed a way to understand the process of stress (Figure 1).

- Individuals, for the most part, try to keep their thoughts, emotions and relationships with the world in a "steady state".

- Each factor of a person's emotional and physical state has a "range of stability', in which that person feels comfortable. On the other hand, when forces disrupt one of these factors beyond the range of stability, the individual must act or cope to restore a feeling of comfort.

- An individual's behaviour aimed at maintaining a steady state makes up his or her "adjustment" process" or coping strategies.
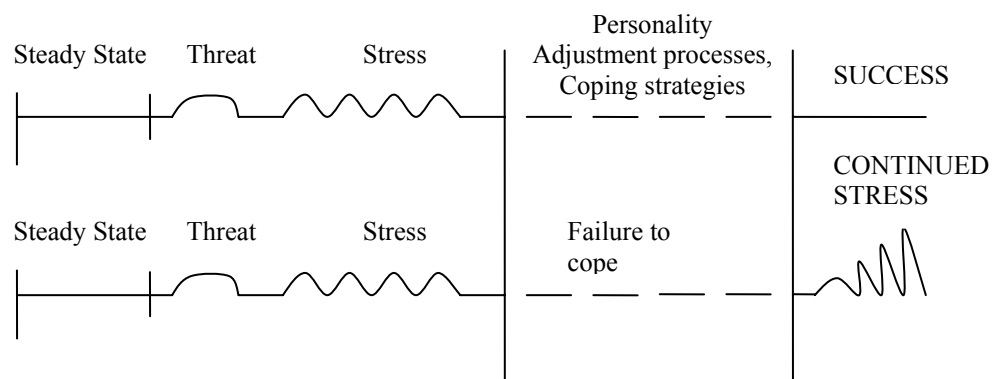
Figure 1. The Cooper-Cummings Framework

Source: **Cary L. Cooper, Rachel D. Cooper, Lynn H. Eaker**
Living with stress. London, England ; New York, N.Y., USA : Penguin Books, 1988

Lazarus and Folkman indicated that an individual is "stressed" when his/her resources are exceeded (Lazarus and Folkman, 1984).

Thus, the stress process involves the individual, availability of resources and one's coping strategies. If the exposure to the stressor is prolonged and one's adjustment is inadequate for adaptation, it would be harmful to the body.

2.2 Moderating factors of stress

2.2.1 Type A behaviour

What stresses one person may have no effect on others. Considerable attention has been given to the individual differences and stress and one of the dimensions in individual differences is Type A behaviour.

Type A behaviour being often referred to as coronary-prone behaviour, was first noted and described by two cardiologists, Ray Rosenman and Meyer Friedman. They noted that those who had this type of behaviour had high incidence of coronary disease. It is a style of behaviour and not specifically a personality style. Therefore, it is actually defined as a way of acting (Allen, 1983).

The typical Type A personality has the following characteristics: competitive, verbally aggressive, hard-driving, unable to relax, very time conscious, easily angered and hostile (Blonnna, 1996).

Several theories had been offered to explain the association of the Type A behaviour pattern with stress. One of them suggested that the Type A pattern was aimed at maintaining control over the environment (Jones et al., 1986). However, other studies showed that Type-A persons experienced more stressful life events and indicated that Type-A persons reported more uncontrollable life events than Type-B persons did (Dimsdale et al., 1978; Suls et al., 1979). Nonetheless, a study of 238 medical students at Seth G S Medical College, India, reported that stress was not found to be

significantly higher in students having Type-A behaviour compared to those with Type-B behaviour (Supe, 1998).

Students from the University of Pennsylvania who were more Type A spent more time studying and had higher grade point averages. In contrast, Type A students were not more successful in relationships with the opposite sex or in social relationships generally (Waldron et al., 1980). A study from Japan reported that their Type A females experienced more stressful life events than their Type A male counterparts (Maruyama and Morimoto, 1997).

A laboratory experiment on the association of the Type A behaviour with reactivity of secretory immune function to a brief stress suggested that Type A personnel might be chronically higher in mucosal immune functioning, but have less immune reactivity to a brief stress (Ohira et al., 1999). It was suggested that there was a positive causal relationship between Type-A behaviour and high level of plasma cortisol (LeBlanc and Ducharme, 2005).

It has also been suggested that the display of Type A behaviour depends in part of the socialisation process (Knight, 1981). Dental training is well-documented as a stressful process. Students are expected to master a large volume of new information, learn to cope with demands of adapting to a new living environment, new peers, academic pressure, or sexual concern, and not have enough time to do assigned course work, study, and relax. Thus, the dental school environment may provide conditions that elicit the display of Type A behaviour.

2.2.2   Coping strategies

As indicated in the Cooper-Cummings framework, the way an individual copes with a stressor, once he/she has perceived it as such, becomes an integral part of his/her "vulnerability profile". Inappropriate coping strategies may actually add to the stress experienced; the desire to deal with a threat inappropriately. Research indicated that style of coping is one moderating influence in the stress-distress relationship (Folkman et al., 1986).

A meta-analytic review on coping strategies revealed that men and women have different styles of coping. Women are more likely than men to engage in most coping strategies. Generally, women are more likely to use strategies that involved verbal expressions to others or the self – to seek emotional support, ruminate about problems, and use positive self-talk. Women also have the tendencies to appraise stressors as more severe (Tamres et al., 2002). In addition, people who habitually utilise unhealthy coping strategies such as ignoring problems in the hope that it will go away are more vulnerable to stress (Fones, 1996)

Many university students perceive education as stressful and find it difficult to cope and require or enlist support in various ways. The grade level achieved and whether they drop out, or continue, depends partly on how they cope with pressure at university and the support that is given to them or arranged by them (Waugh, 2003). Nelson et al reported that graduate students with higher grade point average were likely to utilise coping style characterises by less denial, more religious coping, more focus on and venting emotions, and more seeking of emotional social support (Nelson

et al., 2001). In Moffat et al's study, students in Glasgow University Medical School, UK, generally used active coping strategies when they faced stressful situations (Moffat et al., 2004). A study by Smith and Dust revealed that religion was one of the important factors for African-American college students in the context of coping with stress (Smith and Dust, 2006). Pau et al explored how dental undergraduates with different levels of emotional intelligence (EI) coped with stress. It was noted that high EI students were more likely to adopt reflection and appraisal, social and interpersonal, and organisation and time-management skills. Low EI students were more likely to engage in health-damaging behaviours in coping (Pau et al., 2004). This may suggest that differences in personal factors, social and family background would influence one's coping strategies.

Generally, persons who actively coped with certain kinds of life events were more likely to have better mental health and immune function (Billings and Moos, 1981; Esterling et al., 1993; Goodkin et al., 1992a; Goodkin et al., 1992b; Molassiotis et al., 1997). In contrast, those who adopted avoidance coping such as denial and disengagement, generally had increased distress and poorer mental health (Carver et al., 1993; Stanton and Snider, 1993) and poorer immune function (Goodkin et al., 1992b). However, the relationship of coping strategies with mucosal immunity and the endocrinological process is still largely unknown.

2.2.3   Social support

Social support is one of the important factors to determine the success of a person's ability to deal with life events and environmental stressors, especially the support provided by family and friends (Holahan and Moos, 1986; Seeman, 1996; Thoits, 1995). Besides its buffering effect against stress, it is considered to influence health directly. Social support affects health in three ways: i) by regulating thoughts, feelings and behaviours to promote health; ii) by fostering an individual's sense of meaning in life; and iii) by facilitating health-promoting behaviours. (Bovier et al., 2004; Wang et al., 2003).

Social support is defined as information leading the subject to believe that he is cared for and loved, esteemed, and a member of a network of mutual obligation. In his review, Cobb concluded that adequate social support can protect people in crisis from a wide variety of pathological states: from low birth weight to death, from arthritis through tuberculosis to depression, alcoholism, and the social breakdown syndrome. Furthermore, social support may reduce the amount of medication required, accelerate recovery, and facilitate compliance with prescribed medical regimens (Cobb, 1976).

Studies have reported that social support from within the workplace may have a greater buffering effect on stress than does support from outside the workplace (Haines et al., 1991; LaRocco et al., 1980). It was also suggested that the buffering effects of social support from inside versus outside the workplace differed by gender. Support within the workplace was more important for men, whereas support from family members was more important for women (Holahan and Moos, 1982).

Burk and Bender from the University of Pacific School of Dentistry in the USA found that their first year dental students relied heavily on themselves and on informal advice from peers for their academic problems and they perceived these resources to be very effective (Burk and Bender, 2005). Medical students from the University of Dundee, Scotland indicated that establishment of good relationship between students and tutors was the most important factor contributing to the success of the student support scheme (Malik, 2000). However, the hypothesis that social support acts as a buffer against the adverse effects of stress on students' academic performances was not supported by the study conducted on medical students from University of Illinois College of Medicine at Chicago. The discrepancy of the finding from the hypothesis may be due to the timing of the study conducted, where at the time when the study was conducted not all students had been completed their five major clerkships (Rospenda et al., 1994).

A number of epidemiological studies have also shown that social support is one of the important factors modulating responses to stressors in the psychological, cardiovascular, endocrine and immune systems (Coyne and Downey, 1991; Uchino et al., 1996). Some findings also showed the linkage of social support and endocrine and immune system function. There were suggestions that social support has protective effects on neuroendocrine and immune functioning (Arnetz et al., 1985; Fleming et al., 1982; Kirschbaum et al., 1995).

2.3   Dental environnemental stress

2.3.1   Source of stress in the dental environment

The dental school has been considered as a stressful environment (Benjakul and Cheunarrom, 2000; Humphris et al., 2002; Knudsen, 1978; Rajab, 2001; Sanders and Lushington, 2002; Sgan-Cohen, 1989; Wexler, 1978). Stress arises from academic factors such as the amount of material students need to learn, facing examinations, student-faculty relationship problems, meeting treatment requirements and also in meeting time and scheduling demands. As some dental students may stay away from their family, they may lack immediate support from their family. Most dental students are at an age where heterosexual and social relationships become important to them, and they may face problems in boy-girl relationships. Due to the heavy course work, they may lack time for relaxation, and interaction with friends and family members. Some may feel insecure about their professional future (Benjakul and Cheunarrom, 2000; Bradley et al., 1989; Garbee et al., 1980; Goldstein, 1979; Musser and Lloyd, 1985; Sgan-Cohen, 1989).

A number of studies were carried out to investigate the source of stress and levels of stress of dental students. One instrument commonly used to identify and quantify the perceived source of stress is the Dental Environmental Stress (DES) questionnaire developed by Garbee et al. (Garbee et al., 1980). Due to the differences in social-cultural backgrounds, and personal beliefs and attitudes, students in different countries perceive stressors in their dental environment differently.In the 1980s, two studies used the DES to examine stress at Louisiana State University's School of

Dentistry. The findings from the first study indicated that student-faculty relationships were the primary course of stress for dental students (Garbee et al., 1980), and in the second study it was the inconsistency of instructor feedback (Garbee, 1981). Subsequently, the same instrument was used by Westerman et al (Westerman et al., 1993) at a private Midwestern dental school in North America, the United Kingdom at Manchester Dental School (Heath et al., 1999), Australia (Sanders and Lushington, 1999), Jordan (Rajab, 2001) and Malaysia (Rosli et al., 2005). In these studies, students perceived "examinations and grades" as the top stressor. In Singapore (Yap et al., 1996), students ranked "completing graduation requirements" as the top stressor. In South Africa (Hendricks et al., 1994) "inadequate time for social activities" was perceived as most stressful. Indian dental students perceived their main sources of stress as fear of failing the course or year as well as fear of facing parents after failure (Acharya, 2003). For Greek dental students, assigned workload, performance pressure, and self-efficacy beliefs constituted the most stress-provoking factors (Polychronopoulou and Divaris, 2005). The most important stressors for Nigerian dental students were those related to the lack of provision of a well-supported system of dental education in Nigeria in terms of availability of materials for clinical training and study materials (Sofola and Jeboda, 2006).

2.3.2 Variables that influence the stress levels

Perceived stress levels were found to be higher among dental students than in general population (Pau and Croucher, 2003). The study of Humphris et al also found that emotional exhaustion was higher in dental undergraduates than in medical

undergraduates (Humphris et al., 2002). It was suggested that age, gender, relationship status, personality type, availability of support and academic year were the variables that explained the variation in stress experienced by dental students.

The clinical years were found to be more stressful than the preclinical years (Henning et al., 1998; Naidu et al., 2002; Newton et al., 1994; Sanders and Lushington, 1999). Female students generally perceived more stress than male students (Goldstein, 1979; Rosli et al., 2005; Sanders and Lushington, 1999; Westerman et al., 1993), and were more stressed about personal factors (Al-Omari, 2005). Students with Type A behaviour reported higher stress in the course of their dental studies (George et al., 1987) and students who were categorised as having tendencies toward practical thinking and independence reported lower levels of stress (Mozer et al., 1990). Those who resided at home during term-time appeared to be less stressed (Humphris et al., 2002). A study on black dental students in South Africa reported that non-academic factors appeared to be quite stressful to the students despite the fact that tests and examinations remained as stressful factors to them (Hendricks et al., 1994). The problems at home caused more stress for students in the study conducted at the University of North Carolina School of Dentistry. (Sturdevant et al., 1987). Availability of student tutor programs was found to be able to reduce the effects of dental environmental stress on the dental students (Rhodes and Swedlow, 1983).

2.3.3   Effect of stress on dental students

Reports have indicated that dental education may results in deleterious consequences. Students suffer high levels of stress which may lead to alcohol and drug abuse

(Newbury-Birch et al., 2002), anxiety and mental exhaustion (Humphris et al., 2002), impair learning efficiency (Tisdelle et al., 1984), result in poorer performance (Cecchini and Friedman, 1987; Westerman et al., 1986) and increased personal problems (Brown and Barnett, 1983), and physical complaints (Wexler, 1978).

Professional school is, for most students, a stress-provoking experience that place considerable demands on the students which is unavoidable (Burk and Bender, 2005). Identification of potential stressors and their impact on students will help the students, and faculty to find appropriate approaches towards student stress. (Al-Omari, 2005; Westerman et al., 1993).

However, most of the studies about perceived sources of stress and stress levels in the dental school environment were descriptive and used subjective methods. There is a need to find a more objective way to complement the subjective assessment of stress. Using a biological indicator may accomplish this objective.

2.4 Salivary biomarkers of stress

There has been increased interest in using biomarkers as a complement to questionnaire based measures for stress assessments. Saliva is one of the important biological materials in biomarkers research. The saliva sample has many advantages compared to other bodily fluids. A major advantage is that the collection of saliva is non-invasive, and generally much preferred by individuals. Thus, repeated sampling for serial measurement is possible. The collection is stress free and safe, which are important for the assessment of psychological stress. There is also a potential for cost saving as it does not require a trained phlebotomist to perform the collection as compared to blood sampling. Moreover, many biomarkers in saliva can represent the level in the serum. However, there are some shortcomings for saliva sample too. The salivary flow rate and circadian rhythm variation may affect many parameters in saliva; and some diseases and medications affect the results too (Stone et al., 1987; Stone et al., 2001). Therefore, appropriate study design and choice of saliva markers are important factors to determine the validity of the study results.

2.4.1 Salivary Immunoglobulin A and stress

Immunoglobulins (Ig) in human saliva were identified more than 40 years ago by Ellison, Mashimo, and Mendel (Ellison et al., 1960), and the presence of IgA was demonstrated by Tomasi and Zeigelbaum in 1963 (Tomasi and Zigelbaum, 1963). The dominant antibody in the secretory (mucosal) immune system of mammals is an IgA isotype, and analysis of immune response in saliva may be used to represent the functional status of the entire mucosal immune system. In saliva, IgA exists as a dimer whereas in serum, IgA occurs predominantly as monomer (Mestecky, 1993).

The secretory process of IgA has been summarised as the following (Tomasi, 1994; Tsujita and Morimoto, 1999). First, in the lamina propria of mucosal membranes or in the connective tissue of glands, IgA producing plasma cells locally produce the dimeric IgA molecules, which are joined by a glycoprotein named the J-chain. Then, part of the dimeric IgA molecules diffuses through basement membranes to the basolateral surface of epithelial cells, where epithelial cells take them up via polymeric immunoglobulin receptors (poly-Ig receptor). After that, these dimeric IgA molecules are transcytosed to the apical surface of the epithelial cells, and released into secretory fluids in the form of secretory IgA (sIgA). This secretory process is shown in Figure 2.

Tsujita and Morimoto (1999) had postulated the possible mechanisms that underlie the variation of sIgA caused by stress. Firstly, since sIgA in saliva is produced locally by plasma cells in the salivary gland, chronic psychological stress decreases salivary sIgA secretion through a reduction in the recruitment of precursor cells to the salivary gland. Secondly, psychological stress or relaxation may possibly modulate plasma

cell activity through stimulation of nerves, cytokines, and hormones, influence the immunoglobulin production by plasma cells. Thirdly, psychological stress or relaxation can affect sIgA transportation by the secretory component (SC)-dependent system in the salivary gland through neural and/or endocrine mechanisms. They divide the IgA responses after stress as a delayed stress effect and an immediate stress effect. The immediate stress effect seems to fade away in a few hours after stress, resulting in a return of increased sIgA to its initial level. While the delayed stress effect may be inhibitory to salivary IgA production several days after stress (Tsujita and Morimoto, 1999).

Figure 2. Selective transport system of IgA into external secretion
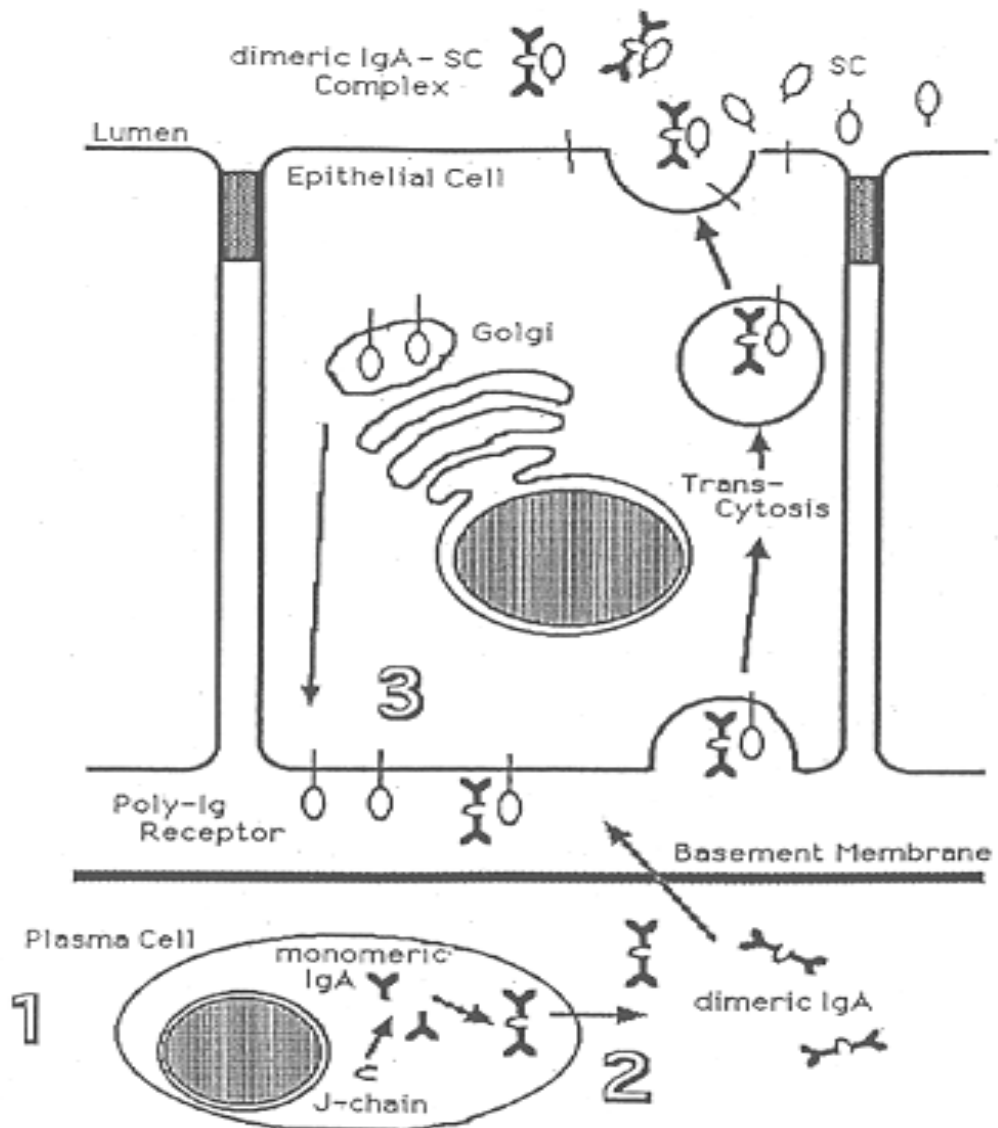
Psychological factors could affect sIgA response at the following three steps in the SC-dependent transport system: 1. Number of plasma cells. 2. Immunoglobulin producing activity of plasma cells. 3. Expression of poly-Ig receptors or SCs.

Source: Tsujita S, Morimoto K.
           Secretory IgA in saliva can be a useful stress marker. *Environ Health Prev Med* 1999;4:1-8.

The effects of stress on the immune system are well documented. One such effect is the suppression of the IgA production in saliva. Various studies have suggested that chronic psychological stress could lead to impairment of immune function, leaving the individuals exposed to greater risk of infections and illness. Salivary IgA secretion rate has been shown to be sensitive to psychological variables such as feelings of loneliness, disgust, depression and power motivation (Evans et al., 1994; Evans et al., 1993; Jemmott et al., 1983; McClelland et al., 1980; McClelland et al., 1985; Miletic et al., 1996).

Salivary sIgA has been studied as a biomarker of job stress level. Workers with a higher level of job stress had a significantly decreased salivary IgA concentration (Henningsen et al., 1992). In one study, female nurses who perceived higher levels of work-related stress had a significantly lower level of salivary IgA concentration and secretion rate than those nurses who perceived lower levels of stress (Ng et al., 1999), while a subsequent study, using a specific questionnaire for health care profession, also showed salivary IgA to be inversely correlated with the nurses self-reported stress (Yang et al., 2002).

Salivary IgA is particularly appealing as a potentially useful biomarkers of stress because (1) it can be obtained non-invasively, easily, and frequently, (2) it is biologically relevant as a functional immune end point, (3) it can be quantified by use of rapid and simple methods such as a radio-immunoassay or enzyme-linked immunoassay, and (4) it is relatively stable, with a biological half-life of 3 to 6 days (Henningsen et al., 1992; Miletic et al., 1996). Lastly, storage of saliva samples at -

30º C for up would not affect to three months salivary IgA concentrations (Ng et al., 2003b)

2.4.2   Salivary lysozyme and stress

Lysozyme, or muramidase, is a mucolytic enzyme widely distributed in human tissues and secretions (Moutsopoulos et al., 1980). It is considered to belong to a primitive defense system known as the innate immune system. It has enzymatic activity, which cleaves beta-1,4 glycosidic bonds between muramic acid and *N*-acetylglucosamine residues in the peptidoglycan of the bacterial cell wall (Jolles and Jolles, 1984; Yeh et al., 1997). Other proposed antimicrobial activities of lysozyme include inhibition of bacterial growth, metabolism and de-chaining (Schenkel LC, 1995). A study on storage effects of salivary biomarkers found that salivary lysozyme concentrations are stable for up to 3 months when stored at -30 ºC (Ng et al., 2003b).

Perera and co-workers studied salivary lysozyme as a biomarker of stress in students during undergraduate examinations and changes in salivary lysozyme levels after exposure to various relaxation strategies. Their findings indicated that lysozyme was sensitive to psychological changes (Perera et al., 1998; Perera et al., 1997). In another study, no significant negative relationship between salivary lysozyme and levels of self-perceived stress was found (Ng et al., 1999). However, in a subsequent study, using a specific questionnaire for healthcare professionals, a negative relationship between salivary lysozyme and levels of self—perceived stress was noted (Yang et al., 2002). Furthermore, explanations accounting for the observed association of

lysozyme levels with stress are still conjectural and need further elucidation (Peretz et al., 1997). Therefore, further study is merited to assess salivary lysozyme as a possible stress biomarker.

2.4.3   Salivary cortisol and stress

Cortisol is a lipophilic steroid with low molecular (MW ~362 Daltons). It is synthesized in the adrenal cortex and released into the blood stream. Up to 95% of the secreted cortisol is bound to large proteins such as albumin and carried throughout the body in the blood. Its activity relies on binding to its glucocorticoid receptors in the cells. Only a small fraction is unbound, which is thought to be biologically active. Due to its low molecular weight and lipophilic nature, the unbound cortisol enters cells by passive diffusion, which makes it feasible to measure the free cortisol fraction in all body fluids (Vander et al., 2001).

Cortisol secretion increases in response to any stress in the body, whether it is physical (such as illness, trauma, surgery, or temperature extremes) or psychological stress. It is often regarded as a "stress hormone".

It is now widely accepted that psychological stress can increase the activity of the hypothalamic-pituitary-adrenal (HPA) axis with subsequent rise in cortisol levels (Schulz *et al.* 1998). Although there is considerable evidence that acute stressors increase cortisol secretion (Kirschbaum and Hellhammer, 1994; Ng et al., 2003c), it is less clear what the effects of chronic stress are on cortisol. Zeier had found that the working sessions caused a marked increase in the concentration of salivary cortisol, and also salivary cortisol response was correlated with workload measures (Zeier *et*

al. 1996). Chronically stressed subjects showed an enhanced and prolonged increase of cortisol level after awakening compared to non-stressed subjects (Schulz *et al*. 1998). However, others (Margit *et al*. 1995) have found no evidence that chronic stress associated with unemployment affects overall salivary cortisol excretion. Lately, there has been some studies that has shown that chronic stress results in lowered cortisol (saliva and plasma). It is thought that is due to down-regulation of the HPA axis from chronic stimulation of chronic stress (Boscarino 1996; Pruessner, Hellhammer et al. 1999; Yang, et al. 2001). Thus, more work is needed to clarify the relationship between cortisol and stress, especially chronic stress.

In recent years, salivary cortisol has become a popular measure of HPA activity in stress research for several reasons. First, plasma unbond cortisol and salivary cortisol correlate highly (correlation coefficient, r >0.90) (Kirschbaum and Hellhammer, 1994). Second, the time lag between changes in plasma cortisol and salivary cortisol is very short (1-2 minutes) (Sapolsky, 1999). Third, saliva flow rate has no impact on salivary cortisol levels (Kirschbaum and Hellhammer, 1994). Finally, saliva samples collection would not impose any stress to subjects, whereas plasma cortisol findings may be biased by venesection stress (Kirschbaum and Hellhammer, 1994; Meeran et al., 1993). A study has shown that salivary cortisol is stable at room temperature for about a week and up to 3 months at 5º C for at least 1 year at -20 ºC or -80º C (Garde and Hansen, 2005).

CHAPTER 3

AIMS AND OBJECTIVES

The main aim of this research is to study the stress patterns of a population of dental undergraduates throughout the academic year as well as to study the moderating effect of personality, coping strategies and social support on stress levels. In addition, it is also to investigate the relationship of salivary biomarkers with stress.

The objectives of the study were:

3.1   To assess the sources of stress and stress levels of dental undergraduates using:

3.1.1   Dental Environnemental Stress (DES) questionnaire

- To assess and compare the academic stressors at different times of the academic year (beginning, middle and the end) for

  - male and female dental undergraduates.

  - dental undergraduates from different academic classes

- To identify the ranking of the academic stressors of dental undergraduates based on DES subscales at different times of the academic year (beginning, middle and the end).

3.1.2   General Life Events (GLE) questionnaire

- To assess and compare the non-academic stressors at the different times of the academic year (beginning, middle and the end) for

  - male and female dental undergraduates.

  - dental undergraduates from different academic classes.

3.1.3   Perceived Stress Scale (PSS)

- ▪ To assess and compare the self-perceived stress at different times of the academic year (beginning, middle and the end) for
  - • male and female dental undergraduates.
  - • dental undergraduates from different academic classes.

3.1.4   General health questionnaire (GHQ)

- ▪ To assess and compare the impact of stress on normal "healthy" functions using GHQ at the different times of the academic year (beginning, middle and the end) for:
  - • male and female dental undergraduates.
  - • dental undergraduates from different academic classes.

3.1.5   To investigate the relationship of stressors (academic and non-academic) and stress levels with GHQ.

3.2   **To study factors that may modify the stress response among dental undergraduates.**

- ▪ To assess and compare modifying factors of stress [(i). personality type, (ii) Coping strategies and (iii) Social support] at the beginning and the end of the academic year for:
  - • male and female dental undergraduates.
  - • dental undergraduates from different academic classes.

3.2.1   To investigate the relationships of the different modifying factors with the PSS and GHQ.

3.3   To study salivary biomarkers of stress among dental undergraduates.

3.3.1   Salivary biomarkers of stress

- To assess and compare the different salivary biomarkers of stress [(i) cortisol, (ii) IgA and (iii) lysozyme] at different times of the academic year (beginning, middle and the end) for

  - male and female dental undergraduates.

  - dental undergraduates from different academic classes.

3.3.2   To investigate the relationship between stressors (academic and non-academic) with salivary biomarkers.

3.3.3   To investigate the relationship of different modifying factors (personality, social support and coping strategies) with salivary biomarkers.

CHAPTER 4

MATERIALS AND METHODS

4.1   Study design

This was a longitudinal study for a period of one year from August 2002 to July 2003. Administration of questionnaire and salivary collection were carried out at the beginning, middle and end of the academic year.

At the middle of the academic year, questions for modifying factors of stress (type-A personality, coping strategies and social support) were not included. We believe that those factors would not have changed in a short period of time.

4.2   Study population

Dental Undergraduates

All 1st to 4th year dental undergraduates of academic year 2002/2003 from the Faculty of Dentistry (FoD) in the National University of Singapore (NUS) were eligible for the study. Currently, NUS is the only institution that offers an undergraduate course in dentistry in Singapore.

4.2.1   Criteria of inclusion of the subjects

All dental undergraduates of NUS in academic year 2002/2003 were invited to participate in the study.

4.2.2   Criteria of exclusion of subjects for salivary biomarkers analyses

To ensure the validity of the results, the exclusion criteria for participation were:

A. Pregnancy, as pregnancy might affect the subjects' immunity and hormone levels.

B. Chronic diseases affecting the immune system.

C. Regular medication with known effects on the immune system and cortisol profile such as corticosteroids and sublingual hormones.

D. Present or past (one week) history of upper respiratory tract infection (URTI), as URTI would affect the levels of IgA, lysozyme and cortisol in the saliva.

E. The flow rate of saliva of valid subjects should not be less than 0.1 ml/min (under basal conditions, the average rate of saliva production is 0.5ml/min) (Guyton, 1991). Subjects with a flow rate of less than 0.1 ml/min would probably not have collected the saliva properly for the 5-minute period.

4.3   Data collection

4.3.1   Questionnaire

The dental undergraduates were given a self-administered questionnaire (attached in Appendix A). The questionnaire included items of information on:

I.   Personal data: academic year, gender, date of birth, ethic group, nationality, marital status, religion, and type of dwelling arrangement.

II. Personality: A 14-question instrument with a scale of 1 to 11 was used to assess the extent of the individual's Type-A behaviour is present. The higher the score received on this questionnaire, the more firmly an individual can be classified as Type A. Anyone with a score of > 84 is inclined towards Type A behaviour (Cooper et al., 1988)

III. Coping strategies: A 52-item Coping Orientation to Problems Experienced (COPE) scale was used to assess participants' common coping strategies when faced with stressful events (Carver et al., 1989). The COPE consists of 13 4-item subscales, each measuring a different set of coping responses. The 13 subscales are:

1)   Active coping

2)   Planning

3)   Seeking social support for instrumental reasons

4)   Seeking social support for emotional reasons

5)   Suppression of competing activities

6)   Turning to religion

7)   Positive reinterpretation and growth

8)   Restraint coping

9)   Acceptance

10)   Focus on and venting of emotions

11)   Denial

12)   Mental disengagement

13)   Behavioral disengagement

Participants rated themselves on these coping strategies by indicating the frequency of use for each strategy using a 5-point scale ranging from 0 (never) to 4 (very often). Scoring for the COPE scale is done by summing the relevant items to obtain a score for each subscale.

The subscales were further grouped into the following three categories (Carver et al, 1989): A) problem-focused coping, comprising the above scales 1,2,3,5 and 8;) B) emotion-focused coping, comprising scales 4,6,7,9 and 11; and C) less-useful coping, comprising scales 10, 12 and 13. Problem-focused and emotion-focused responses were considered more adaptive than less-useful coping.

The minimum score was 20 and maximum score was 80 for problem-focused coping and emotion-focused coping in COPE. For the less-useful coping, the minimum and maximum score were 12 and 48, respectively. An average score for each category, derived by dividing the total score by the number of scales that comprise it, allows meaningful comparison among the three categories.

IV.   Social support: This was assessed by asking the dental undergraduates about the people in their environment who provided them with help/support when they were in need. The categories of people included: supervisor, mentor, course-mates, family members/friends/relatives, religious groups and others for them to specify. The answer were on 1 = do not have such a person, 2 = not at all, 3 = a little, 4 = some time and 5 = very much. Four (4) and 5 were taken as indicative of positive response.

V.   Life Events: there were two parts of questionnaire for this section so as to measure their sources of stress academically and non-academically

(1)   Life events for dental students: Dental Stress Questionnaire (DES)

This 38-item DES questionnaire developed by Garbee et al (1980) is a common questionnaire used to identify and quantify the perceived stressors in dental environment (Garbee et al., 1980).

They can be grouped into four different subscales: Academic work (AW), Faculty and administrative factors (FF), Clinical factors (CF) and Personal factors (PF). (attached in Appendix B).

The responses are made on a 4-point Likert scale (1= not stressful, 2=slightly stressful, 3=moderately stressful, 4=very stressful, 5 = not applicable). As several items were not relevant to some students, not every item was ranked. Items which had less than 20% responses were labelled as not applicable.

(2)   General Life Events: It was adapted from Cooper, Cooper and Cheng Life Events Scale (Living with stress 1988) with deletion of those items that were not relevant to dental students. This section asked students how upsetting a list of events that they had experienced for the past two months was. The

responses were made on 10-point scale from 1(least upset) to 10 (worst imaginable level of upset). A total score of >50 indicates that individual were adversely upset by such events, and is an indication of a potentially stressful situation.

V.   Self Perceived Stress: Perceived Stress Scale-10 (PSS-10)

The PSS-10 developed by Cohen et al. (Cohen and William, 1988) was used to assess students' self-perceived stress levels. Students indicated how often they had experienced the condition during the past two months based on a 5-point Likert scale (0=never, 1=almost never, 2=sometimes, 3=fairly often and 4=very often). Four of the items (4,5,7,8) are reverse-scored. The total scores are derived by summing up all the items responses and the possible range of scores is 0-40. The test-retest reliability of the scale conducted was 0.86 (Sewitch et al., 2001).

VI.   Heath outcome measure: General Health Questionnaire (GHQ-28):

This questionnaire was originally developed to detect minor psychiatric disorders among respondents in community settings. It was adopted to provide a general measure of psychological well-being. Subjects were asked to indicate whether they had experienced the condition more or less than usual during the past two months. Each item was then recoded according to "the GHQ score" as 0-0-1-1. A GHQ-28 score of ≥ 5 was indicative of psychiatric morbidity. The reliability of the GHQ-28 is satisfactory (Cronbach alpha = 0.81). (Goldberg and Williams, 1988).

The time for students to complete the questionnaires was approximately 15 minutes.

4.3.2   Salivary sample collections

A. A written protocol on how the saliva would be collected was given to the dental undergraduates. (Attached in appendix C)

B. In addition, the dental undergraduates were also briefed and supervised on the collection method on the day of collection.

C. Dental undergraduates were asked not to eat and drink (except water) one hour prior to saliva collection, as food debris could stimulate salivation. A single-timed 5-minute unstimulated saliva was obtained from. The time of saliva collection was between 11 a.m. - 1 p.m., after they finished their morning lectures. This timing was designed to minimise the variation that might be introduced by circadian rhythm.

D. Dental undergraduates were also asked to record whether they had suffered from URTI on the day the saliva was collected or in the past one week.

E. After the collection, the volume of the saliva was measured and divided by five minutes to obtain the salivary flow rate, which was expressed in ml/min.

F. The samples were immediately brought to the laboratory and stored frozen at – 70°C until required for assay.

4.4   Field work

4.4.1   Confidentiality

Confidentiality was emphasised to the participants. The dental undergraduates were not named. The completed questionnaires were given to a field investigator who was not involved in the data management.

4.4.2   Participation

We obtained the approval from the Dean of FoD as well as ethical approval for the study from the Institutional Review Board of the National University Hospital. The participation was strictly voluntary, and informed consent was obtained from each student.

4.4.3   Field work

A field investigator was available to assist with the study. She was educated thoroughly on the saliva collection as well as in the distribution and collection of the data. This minimised observer bias.

4.4.4   Pilot study

A pilot study for the questionnaire was conducted on a small group of 15 dental undergraduates. Minor changes were made for some questions to be more relevant to dental undergraduates. Some grammatical changes were also made.

4.4.5   Supervision

On the day of salivary sample collection, a field investigator together with the author briefed and supervised the dental undergraduates on the proper collection method. Subjects with a flow rate of less than 0.1 ml/min would probably not have collected the saliva properly for the 5-minute period. So re-explaining of the method and asking the dental undergraduates to provide a second 5-minute salivary sample was done. This was to reduce the exclusion rate due to incorrect collection technique.

4.5   Biomarker analyses

4.5.1   Salivary IgA:

Salivary IgA concentration (μg/ml) was determined using Salimetrics HS-IgA Kit (Salimetrics LLC, USA).

4.5.2   Salivary lysozyme

The salivary lysozyme concentration (μg/ml) was measured using an ELISA method that was developed in our laboratory as described in a previous published paper (Yang et al., 2002).

5.5.3   Salivary cortisol

Salimetrics HS-Cort kit (Salimetrics LLC) was used for the quantitative measurement of salivary cortisol (nmol/l).

The salivary IgA and lysozyme secretion rates (µg/min) were computed by multiplying the absolute IgA and lysozyme concentration with the absolute saliva flow rate. Since salivary cortisol is not affected by salivary flow rate, only salivary concentration (nmol/l) was used in the analysis (Kirschbaum and Hellhammer, 1994).

4.6   Quality control

4.6.1   Response rate

Drop out is a major concern to this longitudinal study. Measures were taken to minimise it which included the following:

A. A clear explanation was made to all the dental undergraduates about the significance of the study.

B. It was emphasised that the study would provide a basis for improvements in dental education environment and provide data for future students, and faculty to find appropriate approaches toward dental students stress management.

C. Checking the questionnaires after collected for some questions that the dental undergraduates may have forgotten to answer.

4.6.2   Quality control method

Quality control was important for the validity of the results observed. Some measures were used as the following:

A. Use of validated instruments wherever possible and appropriate.

B. Interviewing a random selection of dental undergraduates to determine whether they had been told about the objective and significance of the study.

4.7   Data processing and analysis

A. Before the analysis of the data, a thorough check of the data and correction of any mistakes were done during data entry to minimise the errors.

B. Throughout this report, means and confidence intervals and proportions of the dental undergraduates who had positive responses (in %) are used to present the data; all the confidence intervals and p values were two sided.

C. For those questionnaires where the responses were made on the Likert scale, parametric methods were used for data analysis. It was based on the assumption that there were equal intervals for the response scales, and an equal weightage for all the questions.

D. An independent-samples t-test was used to assess the differences of DES, PSS, COPE and salivary biomarkers between male and female dental undergraduates.

E. One-way Analysis of Variance was used to compare the differences of DES, PSS and COPE scores among the dental undergraduates from the four academic classes.

F. General linear model (GLM) was used to analyse the factors such as gender which may affect the salivary biomarkers results.

G. The Pearson or Spearman's correlation coefficients was calculated to assess the relationship between different stress scores and different biomarkers where

applicable, depending on whether parametric or non-parametric tests have been regulated.

H. Chi-Square test was used to analyse the differences of distributions on GLE, GHQ and personality scores between male and female dental undergraduates and also dental undergraduates across the four academic classes.

I. The Chi-square trend test was used to test the trend on distribution of GHQ scores of > 5 from the beginning to the end of the academic year.

J. The level of statistical significance was considered to be the conventional alpha = 0.05.

K. The data was analysed using SPSS-PC version 13.0 (SPSS 13.0, 2005).

CHAPTER 5

RESULTS

5.1 Response rates and valid subjects

5.1.1 Beginning of the academic year

All the 134 dental undergraduates (100%) completed the questionnaire. **Salivary biomarkers** – Based on the selection criteria, 110 (82.1%) dental undergraduates were included for the salivary biomarkers analyses. The valid subjects for the salivary biomarkers analyses according to distribution by dental class were: number (n)=28 (82.4%) for 1st year, n=26 (74.3%) for 2nd year, n=27 (84.4%) for 3rd year and n=29 (87.9%) for 4th year class. The total number of valid subject was 110 (82.1%).

5.1.2 Middle of the academic year

All except one 3rd year dental undergraduate completed the questionnaire. The response rate was 99.3%. **Salivary biomarkers -** The valid subjects according to distribution by dental class were: n=25 (73.5%) for 1st year, n=25 (71.4%) for 2nd year, n=23 (71.9%) for 3rd year and n=23 (70.0%) for 4th year class. The total number of valid subjects was 96 (71.6%).

5.1.3 End of the academic year

All except two of the 2nd year dental undergraduates (98.5%) completed the questionnaire. **Salivary biomarkers -** The valid subjects according to the distribution by dental class were: n=26 (76.5%) for 1st year, n=24 (68.6%) for 2nd year, n=27

(84.4%) for 3rd year and n=29 (87.9%) for 4th year class. The final number of valid subjects for salivary biomarkers analyses was 106 (79.1%).

Analysis to compare self-perceived stress levels of the group of students having URTI with those not having URTI showed that there was no significant difference in term of the stress levels.

5.2   Socio-demographic characteristics of all the dental undergraduates (n=134)

There were 83 males and 51 females in this study cohort. They were aged between 19 to 25 years old. The majority of them were Chinese (92.5%). There were only six Indians (4.5%), three Malays (2.2%) and one other ethnic group (0.8%) in this study cohort. Singaporeans constituted 82.1% in this cohort while the remaining 17.9% were Malaysians. More than one third of the dental undergraduates were Christian (39.1%), followed by 29.1% who were Buddhist and 23.1% who were of no religion. Most of them (77.6%) stayed in their parent's house or own house, 20.1% of them stayed in the hostel, two of them stayed at a relative's place and one stayed in a rented room. The socio-demographic characteristics of all the dental undergraduates are presented in Table 5.1.

Table 5.1. Demographic characteristics of all the dental undergraduates (n=134)

Characteristic	Year 1 (n = 34)	Year 2 (n = 35)	Year 3 (n = 32)	Year 4 (n = 33)	Overall (n = 134)
Gender					
Male	22	17	21	23	83
Female	12	18	11	10	51
Age (years)					
Mean ± SD	20.5 ± 1.1	21.3 ± 1.2	22.3 ± 0.9	23.0 ± 1.5	21.8 ± 1.4
Min - Max	19 - 23	20 - 24	20 - 23	21 - 25	19 - 25
Ethic group					
Chinese	33	30	28	33	124
Malay	1	0	2	0	3
Indian	0	5	1	0	6
Others	0	0	1	0	1
Nationality					
Singaporean	26	29	26	29	110
Malaysian	8	6	6	4	24
Others	0	0	0	0	0
Religion					
Buddhism	12	12	8	7	39
Christianity	10	12	14	17	53
Islam	1	2	2	0	5
Hinduism	0	2	0	0	2
No religion	11	4	7	9	31
Other religion	0	3	1	0	4
Place of residence					
Parent's house/ Own house	25	26	24	29	104
Hostel	9	7	8	3	27
Rented room	0	1	0	0	1
Relative's place	0	1	0	1	2
Others	0	0	0	0	0

5.3 **Sources of stress and stress levels of dental undergraduates**

5.3.1 **Dental Environmental Stress (DES) questionnaire and its subscales**

5.3.1.1 Beginning of the academic year

Statistically, male-female comparison indicated females had significantly higher scores than males on "atmosphere created by clinical faculty" (mean score, 95% confidence interval for females: 3.37, 3.03-3.50; males 2.86, 2.64-3.01), and "expectations of dental school and what in reality it is like" (females: 2.96, 2.73-3.19; males: 2.62, 2.43-2.82). In contrast, males (1.81, 1.57-2.07) had statistically significantly higher scores on "amount of cheating in dental school" than females (1.41, 1.17-2.04). There were no statistically significant differences on other items of DES questionnaire between males and females.

As shown in table 5.2, there were statistically significant differences ($p<0.05$) on 12 DES questionnaire items across four academic classes. Based on the combined mean scores of DES, 4th year dental undergraduates had the highest scores (2.73), followed by 2nd year (2.61), 3rd year (2.50) and 1st year dental undergraduates (2.27). However, a statistically significant difference was only noted between 4th year and 1st year dental undergraduates (Table 5.2).

Overall, dental undergraduates ranked academic work (AW) with the highest mean scores (2.79), followed by clinical factors (CF) (2.69), faculty and administration factors (FF) (2.61) and personal factors (PF) (2.15). There was no statistically significant difference on all the four subscales of DES between males and females. It

was also noted that 2nd year dental undergraduates had statistically significantly higher mean scores on CF than 1st and 3rd year dental undergraduates (Table 5.3).

Table 5.2. Mean and 95% confidence interval (CI$_{95}$) of dental environmental stress scores of dental undergraduates from different academic classes at the beginning of academic year

Stress Items	Year 1 Mean; CI$_{95}$ (n = 34)	Year 2 Mean; CI$_{95}$ (n = 35)	Year 3 Mean; CI$_{95}$ (n = 32)	Year 4 Mean; CI$_{95}$ (n = 33)	Differences Between Classes	Mean Score Across Classes; CI$_{95}$
• Amount of assigned classwork (#1)	**2.71;** 2.47-2.94	**3.11;** 2.87-3.36	**2.91;** 2.60-3.21	**3.24;** 2.95-3.54	4 > 1	**2.99;** 2.86-3.13
• Lack of cooperation by patients in their home care (#2)	-	-	**2.23;** 1.92-2.54	**3.39;** 3.11-3.67	4 > 3	**2.82;** 2.59-3.06
• Responsibilities for comprehensive patient care (#4)	-	-	**2.91;** 2.58-3.23	**3.36;** 3.13-3.60	4 >3	**3.08;** 2.89-3.28
• Patients being late or not showing up for their appointments (#6)	-	-	**2.59;** 2.22-2.97	**3.48;** 3.18-3.79	4 > 3	**3.03;** 2.78-3.28
• Difficulty in learning clinical procedures (#8)	**2.35;** 1.97-2.73	**3.03;** 2.74-3.32	**2.69;** 2.41-2.97	**2.55;** 2.20-2.90	2 > 1	**2.69;** 2.53-2.85
• Atmosphere created by clinical faculty (#9)	**2.32;** 1.94-2.70	**3.06;** 2.78-3.35	**3.03;** 2.69-3.37	**3.52;** 3.26-3.77	2,3,4 > 1	**3.01;** 2.84-3.18
• Rules and regulations of the school (#17)	**1.50;** 1.21-1.79	**1.64;** 1.39-1.88	**2.00;** 1.68-2.32	**2.34;** 1.98-2.71	4 > 1,2	**1.89;** 1.74-2.05
• Completing graduation requirements (#20)	**2.87;** 2.52-3.22	**2.95;** 2.48-3.42	**3.03;** 2.67-3.39	**3.91;** 3.81-4.01	4 > 1,2,3	**3.25;** 3.08-3.42
• Fear of failing course or year (#25)	**3.06;** 2.77-3.34	**3.12;** 2.78-3.46	**2.68;** 2.31-3.05	**3.67;** 3.41-3.93	4 > 1,3	**3.14;** 2.97-3.30
• Insecurity concerning professional future (#26)	**2.03;** 1.68-2.38	**2.68;** 2.34-3.02	**2.23;** 1.92-2.54	**2.88;** 2.54-3.21	2,4 > 1	**2.45;** 2.28-2.62
• Considering entering some other fields of work (#29)	**1.62;** 1.23-2.01	**1.70;** 1.27-2.13	**2.04;** 1.76-2.32	**2.38;** 1.96-2.80	4 > 1	**1.98;** 1.79-2.17
• Personal physical health (#31)	**1.61;** 1.36-1.86	**1.80;** 1.53-2.07	**1.66;** 1.42-1.89	**2.19;** 1.80-2.59	4 > 1	**1.81;** 1.66-1.95
Overall DES mean and CI$_{95}$.	**2.27;** 2.11-2.43	**2.56;** 2.36-2.77	**2.41;** 2.23-2.55	**2.72;** 2.55-2.89	4 > 1	**2.49;** 2.40-2.58

Post-hoc test (Scheffe) of one-way ANOVA: There were statistically significant differences on 12 DES questionnaire items across four academic classes ($p<0.05$); Overall DES mean for 4th year undergraduates was significantly higher than 1st year undergraduates ($p<0.05$).

Table 5.3. Ranking of dental environmental stress (DES) subscales by different academic classes of dental undergraduates at the beginning of the academic year

Rank	Year 1 (n = 34)	Year 2 (n = 35)	Year 3 (n = 32)	Year 4 (n = 33)	Overall (n = 134)
		Dental Environmental Stress subscales Mean (CI_{95})			
1	AW 2.59 (2.41-2.78)	CF 3.01* (2.74-3.29)	AW 2.64 (2.41-2.87)	AW 2.99 (2.78-3.20)	AW 2.79 (2.68-2.89)
2	CF 2.39* (2.13-2.66)	AW 2.91 (2.68-3.15)	CF 2.51* (2.31-2.71)	CF 2.83 (2.66-3.01)	CF 2.69 (2.57-2.82)
3	PF 1.99 (1.79-2.18)	FF 2.18 (1.96-2.40)	PF 2.18 (1.97-2.38)	FF 2.58 (2.38-2.79)	FF 2.18 (2.08-2.29)
4	FF 1.82 (1.64-2.01)	PF 2.10 (1.89-2.31)	FF 2.16 (1.96-2.37)	PF 2.33 (2.10-2.56)	PF 2.15 (2.04-2.25)

AW: Academic work
CF: Clinical factors
FF: Faculty and administration factors
PF: Personal factors

Post-hoc test (Scheffe) of one-way ANOVA: *2nd year dental undergraduates had significant higher mean scores on CF than 1st and 3rd year dental undergraduates

5.3.1.2   Middle of the academic year

There was no statistically significant difference between male and female dental undergraduates on all the DES questionnaire items.

Analysis of variance indicated that there were statistically significant differences among the four classes on 14 out of 38 DES questionnaires items as shown in Table 5.4. Based on the combined mean scores of DES, 4th year dental undergraduates had the highest scores (2.73), followed by 2nd year (2.64), 3rd year (2.53) and 1st year dental undergraduates (2.18). However, there was no statistically significant difference for all the four academic classes on overall mean score of DES (Table 5.4). Overall dental undergraduates ranked AW with the highest score (mean: 2.86), followed by CF (2.74), PF (2.30) and FF (2.28).

Both males and females had the same ranking order on DES subscales as for overall dental undergraduates and there was no statistically significant difference on those subscales between them. Fourth and 2nd year dental undergraduates scored statistically significantly higher on AW and CF than 1st year dental undergraduates. Fourth and 3rd year dental undergraduates had statistically significantly higher scores on FF than 1st year dental undergraduates. There was no significant difference on PF for all the four classes (Table 5.5).

Table 5.4. Mean and 95% confidence interval (CI$_{95}$) of dental environmental stress scores of dental undergraduates from different academic classes at the middle of the academic year.

Stress Items	Year 1 Mean; CI$_{95}$ (n = 34)	Year 2 Mean; CI$_{95}$ (n = 35)	Year 3 Mean; CI$_{95}$ (n = 31)	Year 4 Mean; CI$_{95}$ (n = 33)	Differences Between Classes	Score Across Classes Mean; CI$_{95}$.
• Amount of assigned classwork (#1)	**2.59;** 2.34-2.83	**3.09;** 2.83-3.34	**3.13;** 2.88-3.37	**3.19;** 2.92-3.45	2,3,4 > 1	**2.99;** 2.86-3.12
• Difficulty of classwork (#3)	**2.58;** 2.28-2.87	**3.12;** 2.89-3.34	**2.97;** 2.69-3.24	**2.85;** 2.60-3.10	2 > 1	**2.88;** 2.75-3.01
• Atmosphere created by clinical faculty (#9)	**2.35;** 1.99-2.71	**3.09;** 2.79-3.39	**2.90;** 2.54-3.25	**3.79;** 3.59-3.98	4 > 1,2,3	**3.07;** 2.90-3.25
• Relations with members of the opposite sex (#10)	**1.70;** 1.41-1.98	**1.73;** 1.43-2.02	**2.43;** 2.07-2.80	**2.00;** 1.68-2.32	3 > 1	**1.95;** 1.79-2.11
• Receiving criticism about work (#11)	**1.91;** 1.69-2.13	**2.63;** 2.35-2.91	**2.48;** 2.19-2.78	**2.70;** 2.37-3.02	2,3,4 > 1	**2.43;** 2.29-2.58
• Lack of confidence to be a successful dentist (#14)	**2.13;** 1.75-2.50	**2.94;** 2.65-3.23	**2.40;** 2.04-2.76	**2.48;** 2.13-2.84	2 > 1	**2.49;** 2.31-2.66
• Amount of cheating in dental school (#16)	**1.15;** 0.98-1.32	**1.41;** 1.15-1.67	**2.27;** 1.96-2.58	**1.91;** 1.56-2.25	3 > 2 > 1	**1.76;** 1.59-1.93
• Rules and regulations of the school (#17)	**1.50;** 1.21-1.79	**1.91;** 1.62-2.20	**2.30;** 1.94-2.66	**2.82;** 2.51-3.13	3 > 1,4 > 1,2	**2.13;** 1.96-2.30
• Completing graduation requirements (#20)	**2.20;** 1.80-2.60	**3.12;** 2.81-3.42	**3.34;** 3.02-3.67	**3.81;** 3.67-3.96	2,3,4 >1, 4>2	**3.17;** 2.99-3.35
• Expectations of dental school and what in reality it is like (#23)	**2.39;** 2.10-2.69	**2.77;** 2.43-3.12	**2.77;** 2.45-3.09	**3.06;** 2.72-3.40	4 > 1	**2.75;** 2.59-2.91
• Lack of input into the decision-making process of the school (#24)	**1.81;** 1.49-2.14	**2.30;** 1.94-2.66	**2.41;** 2.10-2.73	**2.75;** 2.42-3.08	4 > 1	**2.32;** 2.15-2.49
• Insecurity concerning professional future (#26)	**2.12;** 1.78-2.46	**2.82;** 2.51-3.14	**2.33;** 1.98-2.69	**2.66;** 2.32-2.99	2 > 1	**2.48;** 2.32-2.65
• Financial responsibilities (#27)	**2.09;** 1.81-2.38	**2.36;** 2.01-2.72	**2.62;** 2.23-3.01	**2.94;** 2.64-3.23	4 > 1	**2.49;** 2.32-2.66
Inconsistency of feedback on your work between different instructors (#37)	**1.70;** 1.40-2.00	**2.88;** 2.59-3.18	**2.40;** 2.05-2.75	**3.03;** 2.74-3.32	2,3 > 4 > 1 3 > 4	**2.53;** 2.36-2.70
• Fear of being unable to catch up if left behind (#38)	**2.88;** 2.61-3.15	**3.43;** 3.19-3.67	**2.97;** 2.56-3.38	**3.42;** 3.16-3.69	4 > 3, 2 > 1	**3.18;** 3.03-3.33
Overall DES mean and CI$_{95}$	**2.18;** 2.00-2.37	**2.64;** 2.47-2.82	**2.53;** 2.35-2.72	**2.73;** 2.55-2.90	NS	**2.52;** 2.43-2.61

Post-hoc test (Scheffe) of one-way ANOVA: There were statistically significant differences on 14 DES questionnaire items across four academic classes ($p<0.05$); There was not significantly difference for all the four academic classes on overall mean of DES ($p>0.05$).

Table 5.5. Ranking of dental environmental stress subscales by different academic classes of dental undergraduates at the middle of the academic year

	Year 1 (n = 34)	Year 2 (n = 35)	Year 3 (n = 31)	Year 4 (n = 33)	Overall (n = 133)
Rank		Dental Environmental Stress subscales Mean ($CI_{95.}$)			
1	AW 2.56 * (2.34-2.77)	AW 3.07* (2.89-3.26)	AW 2.83 (2.61-3.05)	AW 2.97* (2.77-3.16)	AW 2.86 (2.75-2.96)
2	CF 2.32$^{\#}$ (1.97-2.66)	CF 2.88$^{\#}$ (2.63-3.14)	CF 2.69 (2.49-2.88)	CF 2.92$^{\#}$ (2.72-3.12)	CF 2.74 (2.62-2.86)
3	PF 2.06 (1.88-2.23)	FF 2.37 (2.17-2.57)	PF 2.47 (2.21-2.72)	FF 2.69^{+} (2.49-2.90)	PF 2.30 (2.20-2.41)
4	FF 1.78^{+} (1.60-1.96)	PF 2.28 (2.08-2.49)	FF 2.31^{+} (2.10-2.53)	PF 2.43 (2.21-2.64)	FF 2.28 (2.17-2.40)

AW: Academic work
CF: Clinical factors
FF: Faculty and administration factors
PF: Personal factors

Post-hoc test (Scheffe) of one-way ANOVA:
*4th and 2nd year dental undergraduates had significant higher mean scores on AW than 1st year dental undergraduates ($p < 0.05$)
$^{\#}$4[th] and 2[nd] year dental undergraduates had significant higher mean scores on CF than 1[st] year dental undergraduates ($p < 0.05$)
$^{+}$4[th] and 3[rd] year dental undergraduates had significant higher mean scores on FF than 1[st] year dental undergraduates ($p < 0.05$)

5.3.1.3   End of the academic year

On male-female comparison, a statistically significant difference only noted on "rules and regulations of the school". The mean scores (CI_{95}) of that item were 2.40 (2.15-2.65) and 2.10 (1.85-2.35) for males and females, respectively. Both genders had the highest scores on "atmosphere created by clinical faculty" where the mean scores were 3.25 for males and 3.47 for females.

Table 5.6 shows 14 items of DES that had statistically significant differences across four classes. Based on the combined mean scores of DES, 2nd year dental undergraduates had the highest score of 2.88, followed by 4th year scoring 2.76, 3rd year scoring 2.58 and 1st year dental undergraduates scoring 2.40. However, a statistically significant difference was only noted between 2nd year and 1st year dental undergraduates (Table 5.6).

Table 5.7 shows the ranking of DES subscales for all the four classes of dental undergraduates at the end of the academic year. There was no statistically significant difference on CF and PF for all the four classes. Second year dental undergraduates reported statistically significantly higher scores on AW than 1st and 3rd year dental undergraduates. Statistically, 2nd and 4th years dental undergraduates had significantly higher scores on FF than 1st year dental undergraduates. There was no significant difference between male and female dental undergraduates on the ranking of the DES subscales at the end of the academic year. The ranking order for females was the same as for overall dental undergraduates as shown in Table 5.7. The ranking order for males was CF, AW, FF and PF respectively.

Table 5.6. Mean and 95% confidence interval (CI$_{95}$) of dental environmental stress scores of dental undergraduates from different academic classes at the end of academic year

Stress Items	Year 1 Mean; CI$_{95}$ (n = 34)	Year 2 Mean; CI$_{95}$ (n = 33)	Year 3 Mean; CI$_{95}$ (n = 32)	Year 4 Mean; CI$_{95}$ (n = 33)	Differences Between Classes	Score Across Classes Mean; CI$_{95}$.
• Amount of assigned classwork (#1)	**2.73;** 2.36-3.10	**3.55;** 3.37-3.72	**3.13;** 2.84-3.41	**3.09;** 2.83-3.35	2 > 1	**3.12;** 2.98-3.27
• Difficulty of classwork (#3)	**2.70;** 2.36-3.03	**3.24;** 3.04-3.44	**2.75;** 2.42-3.08	**2.97;** 2.66-3.27	2 > 1	**2.91;** 2.77-3.06
• Competition for grades (#5)	**2.31;** 2.05-2.58	**3.00;** 2.69-3.31	**2.47;** 2.19-2.74	**2.50;** 2.17-2.83	2 > 1,3	**2.57;** 2.42-2.72
• Difficulty in learning clinical procedures (#8)	**2.87;** 2.56-3.17	**3.06;** 2.83-3.29	**2.63;** 2.31-2.94	**2.50;** 2.19-2.81	2 > 4	**2.77;** 2.62-2.91
• Atmosphere created by clinical faculty (#9)	**2.80;** 2.47-3.13	**3.58;** 3.40-3.76	**3.22;** 2.93-3.50	**3.70;** 3.53-3.86	2 > 1,4 > 1,3	**3.33;** 3.20-3.47
• Receiving criticism about work (#11)	**2.44;** 2.18-2.70	**2.97;** 2.73-3.21	**2.69;** 2.42-2.95	**2.56;** 2.27-2.85	2 > 1	**2.66;** 2.53-2.80
• Difficulty in learning precision manual skills (#12)	**2.94;** 2.63-3.25	**3.09;** 2.83-3.35	**2.45;** 2.15-2.75	**2.41;** 2.12-2.71	2 > 3,4	**2.74;** 2.59-2.89
• Lack of confidence to be a successful dentist (#14)	**2.44;** 2.07-2.80	**3.10;** 2.79-3.40	**2.56;** 2.25-2.88	**2.77;** 2.46-3.08	2 > 1	**2.71;** 2.55-2.88
• Amount of cheating in dental school (#16)	**1.24;** 1.02-1.46	**1.85;** 1.51-2.19	**2.22;** 1.89-2.56	**1.97;** 1.56-2.38	3,4 > 1	**1.83;** 1.66-2.01
• Rules and regulations of the school (#17)	**1.76;** 1.43-2.09	**2.42;** 2.08-2.77	**2.22;** 1.87-2.57	**2.75;** 2.38-3.12	4 > 1	**2.28;** 2.11-2.46
• Completing graduation requirements (#20)	**2.58;** 2.18-2.98	**3.44;** 3.21-3.66	**3.19;** 2.84-3.54	**3.13;** 2.82-3.43	2 > 1	**3.11;** 2.95-3.27
• Expectations of dental school and what in reality it is like (#23)	**2.45;** 2.11-2.80	**2.97;** 2.68-3.25	**2.78;** 2.45-3.11	**3.09;** 2.83-3.36	4 > 1	**2.82;** 2.66-2.97
• Fear of failing course or year (#25)	**2.79;** 2.41-3.18	**3.03;** 2.70-3.36	**2.81;** 2.45-3.17	**3.63;** 3.39-3.86	4 > 1,3	**3.06;** 2.89-3.23
• Lack of time to do assigned school work (#28)	**2.64;** 2.32-2.95	**3.28;** 3.03-3.53	**3.09;** 2.80-3.39	**2.74;** 2.46-3.03	2 > 1	**2.94;** 2.79-3.08
Overall DES mean and CI$_{95}$	**2.40;** 2.19-2.60	**2.88;** 2.72-3.04	**2.58;** 2.38-2.77	**2.76;** 2.60-2.91	2 > 1	**2.65;** 2.56-2.74

Post-hoc test (Scheffe) of one-way ANOVA: There were statistically significant differences on 14 DES questionnaire items across four academic classes ($p < 0.05$); Overall DES mean for 2nd year undergraduates was significantly higher than 1st year undergraduates ($p < 0.05$)

Table 5.7. Ranking of dental environmental stress subscales by different academic classes of dental undergraduates at the end of the academic year

	Year 1 (n = 34)	Year 2 (n = 33)	Year 3 (n = 32)	Year 4 (n = 33)	Overall (n = 132)
Rank		Dental Environmental Stress subscales Mean ($CI_{95.}$)			
1	CF 2.85 (2.54-3.16)	AW 3.18* (3.02-3.35)	AW 2.76* (2.51-3.01)	AW 2.96 (2.81-3.10)	AW 2.89 (2.78-3.00)
2	AW 2.66* (2.41-2.91)	CF 3.05 (2.89-3.20)	CF 2.69 (2.46-2.93)	CF 2.73 (2.51-2.95)	CF 2.83 (2.72-2.95)
3	PF 2.25 (2.06-2.45)	PF 2.62 (2.39-2.85)	PF 2.43 (2.22-2.65)	FF 2.68^{+} (2.48-2.88)	PF 2.47 (2.37-2.57)
4	FF 2.13^{+} (1.92-2.33)	FF^{+} 2.60^{+} (2.41-2.78)	FF 2.40 (2.21-2.60)	PF 2.58 (2.38-2.78)	FF 2.45 (2.35-2.55)

AW: Academic Work
CF: Clinical Factors
FF: Faculty and Administration Factors
PF: Personal Factors

Post-hoc test (Scheffe) of one-way ANOVA:
*2nd year dental undergraduates had significant higher mean scores on AW than 1st and 3rd year dental undergraduates ($p < 0.05$)
+2nd and 4th year dental undergraduates had significant higher mean scores on FF than 1st year dental undergraduates ($p < 0.05$)

5.3.2.   General Life Events (GLE)

There were no statistically significant differences between male and female dental undergraduates as well as among the four academic classes in the distribution of total GLE scores of > 50 points. Using a total GLE scores of > 50 points to indicate stressful life events, overall, 6.7 %, 1.5% and 1.5% of dental undergraduates had scores > 50 at the beginning, middle and the end of the academic year, respectively (Table 5.8).

Table 5.8. Distribution of total general life events (GLE) score of > 50 by gender and different academic classes and of dental undergraduates at the different times of the academic year

	Total general life events score >50		
	Beginning of the year (T1) N (%)	Middle of the year (T2) N (%)	End of the year (T3) N (%)
Gender			
Male T1: (n = 83) T2: (n = 83) T3: (n = 82)	7 (8.4)	2 (2.4)	1 (1.2)
Female T1: (n = 51) T2: (n = 50) T3: (n = 50)	2 (3.9)	0 (0.0)	1 (2.0)
Academic Classes			
Year 1 T1: (n = 34) T2: (n = 34) T3: (n = 34)	1 (2.9)	0 (0.0)	0 (0.0)
Year 2 T1: (n = 35) T2: (n = 35) T3: (n = 33)	0 (0.0)	1 (2.9)	2 (5.7)
Year 3 T1: (n = 32) T2: (n = 31) T3: (n = 32)	4 (12.5)	1 (3.1)	0 (0.0)
Year 4 T1: (n = 33) T2: (n = 33) T3: (n = 33)	4 (12.1)	0 (0.0)	0 (0.0)
Overall T1: (n = 134) T2: (n = 133) T3: (n = 132)	9 (6.7)	2 (1.5)	2 (1.5)

5.3.3 Self-perceived stress level (PSS)

5.3.3.1 Beginning of the academic year

Female (F) dental undergraduates had statistically significantly higher mean total PSS scores than male (M) dental undergraduates (mean total = 21.37 (F) vs 18.98 (M), p=0.02). There was no statistical significant difference of PSS scores for different academic classes of dental undergraduates. The overall mean total score of PSS for all dental undergraduates was 19.89 (CI_{95}: 18.85 – 20.94).

5.3.3.2 Middle of the academic year

It was noted that female dental undergraduates still had statistically significantly higher PSS scores than male dental undergraduates (22.24 (F) vs 19.92 (M), p=0.04). Based on the academic classes comparison, 4th year dental undergraduates had significantly higher scores of PSS (23.55) than 1st year dental undergraduates (18.97), (p=0.02). The overall total mean of PSS for all dental undergraduates at this time point was 20.79 (19.71 -21.87).

5.3.2.3 End of the academic year

There was no significant difference of PSS score between males and females and also for different academic classes of dental undergraduates. The overall mean score for all the dental undergraduates were 20.35 with a CI_{95} of 19.46 to 21.23.

The details of PSS scores for different academic classes and between male and female dental undergraduates at different times of the academic year were shown in Table 5.9.

Table 5.9. Mean and 95% confidence interval (CI$_{95}$) of perceived stress scale (PSS) score at different times of the academic year

	Mean score of PSS (CI$_{95}$)		
	Beginning of the year (T1)	Middle of the year (T2)	End of the year (T3)
Gender			
Male			
T1: (n = 83)	18.98	19.92	19.86
T2: (n = 83)	(17.52-20.44)	(18.52-21.31)	(18.69-21.04)
T3: (n = 82)			
Female			
T1: (n = 51)	21.37	22.24	21.14
T2: (n = 50)	(20.04-22.71)	(20.56-23.92)	(19.81-22.47)
T3: (n = 50)			
Academic Classes			
Year 1			
T1: (n = 34)	19.62	18.97	18.59
T2: (n = 34)	(17.78-21.45)	(17.31-20.64)	(16.38-20.80)
T3: (n = 34)			
Year 2			
T1: (n = 35)	18.86	20.77	21.61
T2: (n = 35)	(16.82-20.89)	(18.15-23.39)	(19.80-23.42)
T3: (n = 33)			
Year 3			
T1: (n = 32)	18.48	19.87	19.78
T2: (n = 31)	(16.06-20.91)	(17.63-22.11)	(18.50-21.06)
T3: (n = 32)			
Year 4			
T1: (n = 33)	22.61	23.55	21.52
T2: (n = 33)	(20.50-24.71)	(21.58-25.51)	(19.46-21.23)
T3: (n = 33)			
Overall			
T1: (n = 134)	19.89	20.79	20.35
T2: (n = 133)	(18.85-20.94)	(19.71-21.87)	(19.46-21.23)
T3: (n = 132)			

5.3.4   General Health Questionnaire (GHQ)

5.3.4.1   Beginning of the academic year

There were no statistically significant differences in the distribution of total GHQ scores of ≥ 5 between males and females and among the different academic classes of dental undergraduates at three different times of the academic year. However, a higher proportion of females had total scores of ≥ 5 than males. There was also a higher proportion of 4th year dental undergraduates who had total scores of ≥ 5 than other years dental undergraduates throughout the academic year.

Overall there were 46.3%, 56.4% and 63.6% of undergraduates had total scores of ≥ 5 at the beginning, middle and the end of the academic year, respectively. There was an increased trend in the proportion of dental undergraduates who had total GHQ scores of ≥ 5 (Chi-square trend χ^2 =6.8, p=0.009) as shown in Table 5.10.

Table 5.10. Distribution of general health questionnaire (GHQ) 28 total scores of $\geq$5 at different times of the academic year

	Total GHQ 28 score $\geq$5		
	Beginning of the year (T1) N (%)	Middle of the year (T2) N (%)	End of the year (T3) N (%)
Gender			
Male T1: (n = 83) T2: (n= 83) T3: (n= 82)	34 (41.0)	45 (54.2)	47 (57.3)
Female T1: (n = 51) T2: (n = 50) T3: (n = 50)	28 (54.9)	30 (60.0)	37 (74.0)
Academic Classes			
Year 1 T1: (n = 34) T2: (n = 34) T3: (n = 34)	13 (38.2)	17 (50.0)	17 (50.0)
Year 2 T1: (n = 35) T2: (n = 35) T3: (n = 33)	13 (37.1)	18 (51.4)	23 (69.7)
Year 3 T1: (n = 32) T2: (n = 31) T3: (n = 32)	14 (43.8)	16 (51.6)	19 (59.4)
Year 4 T1: (n = 33) T2: (n = 33) T3: (n= 33)	22 (66.7)	24 (72.7)	25 (75.8)
Overall T1: (n = 134) T2: (n = 133) T3: (n = 132)	62 (46.3)	75 (56.4)	84 (63.6)

Chi-square trend for overall total GHQ 28 score of > 5 from T1 to T3: χ^2 =6.8, p=0.009

5.3.4 Relationship of stressors and stress levels with GHQ

The scores of DES and it subscales, GLE and PSS were positively and statistically significantly correlated with GHQ scores at all the different times of the academic year. Correlation coefficients for DES with GHQ at the beginning, middle and the end of the academic year were all 0.49 (p=0.00). Correlation coefficients for GLE with GHQ at the beginning, middle and the end of the academic year were 0.47, 0.44 and 0.26, respectively. The PSS scores were highly correlated with GHQ at different times of the academic year, the r_s were 0.76, 0.72 and 0.67, respectively (Table 5.11).

Table 5.11. Correlation between dental environmental stress questionnaire scores and its subscales, general life events scores and perceived stress scale scores with general health questionnaire scores

	General Health Questionnaire (GHQ) Spearman's rho, (p)		
	Beginning	Middle	End
Dental Environnemental Stress (DES)	0.49, (0.00)	0.49, (0.00)	0.49, (0.00)
Academic Work (AW)	0.44, (0.00)	0.48, (0.00)	0.49, (0.00)
Clinical Factors (CF)	0.33, (0.00)	0.32, (0.00)	0.36, (0.00)
Faculty and Administration Factors (FA)	0.39, (0.00)	0.44, (0.00)	0.40, (0.00)
Personal Factors (PF)	0.47, (0.00)	0.48, (0.00)	0.50, (0.00)
General Life Events (GLE)	0.47, (0.00)	0.44, (0.00)	0.26, (0.00)
Perceived Stress Scale (PSS)	0.76, (0.00)	0.72, (0.00)	0.67, (0.00)

5.4   Modifying factors of stress

5.3.1   Type-A personality

Based on a score of $\geq$ 84 to indicate Type-A personality, results showed that more than three quarters (75.4%) of the dental undergraduates possess a Type-A personality. There were 59 of 83 male (71.1%) and 42 of 51 female (82.4%) dental undergraduates, who had the scores of $\geq$ 84. The distribution of Type-A personality scores of $\geq$ 84 between males and females was not statistically significant different (p=0.142).

5.3.2   Coping strategies (COPE)

5.3.2.1   Beginning of the academic year

Female dental undergraduates had significantly higher scores on emotional-focused coping (F vs M: 9.48 vs 8.67, p=0.02) and less-useful coping (F vs M: 8.19 vs 6.91, p=0.00) than male dental undergraduates.   However, there was no significant difference of various coping strategies adopted by the dental undergraduates from different academic classes.

5.3.2.2   End of the academic year

Female dental undergraduates still had significantly higher score on less-useful-coping than male dental undergraduates (F vs M: 7.74 vs 7.17, p=0.032). In contrast, male dental undergraduates had significantly higher scores on problem-focused coping than female dental undergraduates (M vs F: 9.91 vs 9.31, p=0.027). There was

no significant difference of various coping strategies adopted by the dental undergraduates from different academic classes (Table 5.12).

Table 5.12. Mean and 95% confidence interval (CI$_{95}$) scores of various coping strategies of dental undergraduates by gender and different academic classes at the beginning and the end of the academic year

	Problem faced coping Mean (CI$_{95}$)		Emotional-focused coping Mean (CI$_{95}$)		Less-useful coping Mean (CI$_{95}$)	
	Beginning (T1)	End (T3)	Beginning (T1)	End (T3)	Beginning (T1)	End (T3)
Gender						
Male T1: (n= 83); T3: (n= 82)	10.23 (9.88, 10.57)	9.91 (9.55, 10.26)	8.51 (8.11, 8.90)	8.37 (8.01, 8.73)	6.91 (6.50, 7.32)	7.17 (6.78, 7.56)
Female T1: (n= 51); T3: (n= 50)	9.87 (9.50, 10.24)	9.31 (8.95, 9.66)	9.49 (9.04, 9.93)	8.67 (8.31, 9.02)	8.19 (7.83, 8.55)	7.74 (7.38, 8.10)
Academic Classes						
Year 1 T1: (n= 34); T3: (n= 34)	10.48 (9.94, 11.03)	10.21 (9.80, 10.63)	8.89 (8.32, 9.47)	8.37 (7.79, 8.95)	7.48 (6.93, 8.03)	6.96 (6.38, 7.54)
Year 2 T1: (n= 35); T3: (n= 33)	10.17 (9.66, 10.67)	9.39 (8.94, 9.83)	9.10 (8.42, 9.77)	8.52 (8.06, 8.98)	7.18 (6.49, 7.87)	7.47 (6.99, 7.95)
Year 3 T1: (n= 32); T3: (n= 32)	10.20 (9.64, 10.76)	9.88 (9.23, 10.53)	9.19 (8.56, 9.81)	8.58 (8.05, 9.12)	7.30 (6.60, 7.99)	7.57 (6.83, 8.31)
Year 4 T1: (n= 33); T3: (n= 33)	9.50 (9.05, 9.95)	9.22 (8.66, 9.79)	8.33 (7.74, 8.93)	8.46 (7.88, 9.04)	7.64 (7.05, 8.23)	7.56 (7.11, 8.00)
Overall T1: (n= 134); T3: (n= 132)	10.09 (9.84, 10.35)	9.68 (9.42, 9.94)	8.88 (8.58, 9.18)	8.48 (8.22, 8.74)	7.40 (7.09, 7.70)	7.39 (7.11, 7.66)

5.3.3 Social support

5.4.3.1 Beginning of the academic year

Chi-square test showed that at the beginning of the academic year a higher proportion of female dental undergraduates sought support from "religious group" compared to their male friends (F vs M: 47.1% vs 28%, p=0.04). Other than that, there was no statistical significant difference in the distribution of various types of personnel the dental undergraduates could rely on, either between gender or by academic class. On the whole, a higher percentage of dental undergraduates found that they could easily rely on their family members/friends (85.1%), followed by course-mates (78.2.0%), supervisor (36.6%), religious group (35.3%) and mentor (35.1%) (Table 5.13).

5.4.3.2 End of the academic year

More male dental undergraduates indicated that they could easily talk to their supervisor as compared to female dental undergraduates (M vs F: 48.8% vs 28%, p=0.028). At this time point, a lesser proportion of 4th year dental undergraduates found that they could easily talk to their supervisor and their family members/friends as compared to dental undergraduates in other academic classes (Table 5.13).

On the whole, at the end of the academic year, the trend for the dental undergraduates' social support was fairly similar as at the beginning of the academic year. A higher proportion of dental undergraduates found that they could easily talk to their family members/friends (77.3%), followed by course-mates (75.0.0%), supervisor 40.9%), mentor (37.9%) and religious group (27.7%) when they were in need (Table 5.13).

Table 5.13. Distribution of various social supports of dental undergraduates by gender and different academic years at the beginning (T1) and the end (T3) of the academic year

	Supervisor		Mentor		Course-mates		Family / Friends		Religion Group	
	T1, n (%)	T3 n (%)	T1 n (%)	T3 n (%)	T1 n (%)	T3 n (%)	T1 n (%)	T3 n (%)	T1 n (%)	T3 n (%)
Gender										
Male T1: (n= 83); T3: (n= 82)	31 37.3)	40 (48.8)	31 (37.3)	34 (41.5)	61 (74.4)	60 (73.2)	67 (80.7)	62 (75.6)	23 (28.0)	22 (27.2)
Female T1: (n= 51); T3: (n= 50)	18 (35.3)	14 (28.0)	16 (31.4)	16 (32.0)	43 (84.3)	39 (78.0)	47 (92.2)	40 (80.0)	24 (47.1)	14 (28.6)
Academic Classes										
Year 1 T1: (n= 34); T3: (n= 34)	10 (29.4)	19 (55.9)	16 (47.1)	13 (38.2)	27 (79.4)	29 (85.3)	31 (91.2)	30 (88.2)	9 (26.5)	9 (26.5)
Year 2 T1: (n= 35); T3: (n= 33)	12 (34.3)	15 (45.5)	9 (25.7)	12 (36.4)	29 (85.3)	23 (69.7)	32 (91.4)	29 (87.9)	18 (52.9)	9 (28.1)
Year 3 T1: (n= 32); T3: (n= 32)	16 (50.0)	14 (43.8)	14 (43.8)	14 (43.8)	25 (78.1)	25 (78.1)	26 (81.3)	24 (75.0)	9 (28.1)	6 (19.4)
Year 4 T1: (n= 33); T3: (n= 33)	11 (33.3)	6 (18.2)	8 (24.25)	11 (33.3)	23 (69.7)	22 (66.7)	25 (75.8)	19 (57.6)	11 (33.3)	12 (36.4)
Total T1: (n= 134); T3: (n= 132)	49 (36.6)	54 (40.9)	47 (35.6)	50 (37.7)	104 (77.6)	99 (75.0)	114 (85.1)	102 (77.3)	47 (35.1)	36 (27.2)

5.4.4   Relationship of the different modifying factors with PSS and GHQ scores

The Type-A personality score was positively associated with GHQ (Spearman's rho=0.20, p=0.02) and PSS (Spearman's rho=0.15, p=0.07).

There were negative correlations between problem-focused coping with GHQ and PSS scores. At the beginning of the academic year, the correlation coefficient for problem-focused coping with GHQ and PSS were Spearman's rho=-0.15, p=0.08, and Spearman's rho=-0.25, p=0.00, respectively. At the end of the academic year, the correlation coefficients for problem-focused coping with GHQ and PSS were Spearman's rho=-0.18, p=0.04, and Spearman's rho=-0.20, p=0.05, respectively. There was no significant correlation for emotional-focused coping with GHQ and PSS scores at both the beginning and the end of the academic year. Less-useful coping was positively and statistically significantly correlated with GHQ and PSS scores (correlation coefficients ranged from 0.2 to 0.4, p<0.05) (Table 5.14).

In general, all the different sources of social support were negatively associated with PSS and GHQ score (Table 5.14).

Table 5.14. Correlation between modifying factors of stress with General Health Question (GHQ) and Perceived Stress Scale (PSS)

	Spearman's rho (p)			
	Beginning of the year		End of the year	
	GHQ	PSS	GHQ	PSS
Type A personality score	0.20 (0.02)*	0.15 (0.08)	-	-
Coping Strategies				
Problem-focused coping	-0.15 (0.08)	-0.25 (0.00)*	-0.18 (0.04)*	-0.20 (0.03)*
Emotional-focused coping	-0.08 (0.35)	-0.07 (0.41)	0.07 (0.41)	-0.02 (0.84)
Less-useful coping	0.24 (0.01)*	0.40 (0.00)*	0.20 (0.02)*	0.32 (0.00)*
Social support				
Supervisor	-0.07 (0.43)	-0.04 (0.67)	-0.25 (0.00)*	-0.19 (0.04)*
Mentor	-0.23 (0.01)*	-0.19 (0.03)*	-0.10 (0.27)	-0.09 (0.29)
Course-mates	-0.14 (0.10)	-0.16 (0.07)	-0.13 (0.14)	-0.24 (0.01)*
Family members/Friends	-0.06 (0.51)	0 (1.00)	-0.08 (0.40)	-0.16 (0.07)
Religious groups	-0.07 (0.40)	-0.10 (0.27)	0.21* (0.02)	-0.04 (0.67)
Total social support	-0.18 (0.03)*	-0.17 (0.05)*	-0.06 (0.51)	-0.23 (0.01)*

* $p < 0.05$

5.5 Salivary biomarkers

As the data of the salivary biomarkers were positively skewed, natural logarithmic transformations were performed to normalise the data for further statistical analyses (Figures 5.1 to Figures 5.3).

5.5.1 Comparison on levels of salivary cortisol, IgA and lysozyme at different times of the academic year for male and female dental undergraduates

5.5.1.1 Beginning of the academic year

There were no statistically significant differences on salivary cortisol concentrations and lysozyme secretion rates between male and female dental undergraduates. Nonetheless, females had statistically significantly lower IgA secretion rates (Geometric Mean (GM): 48.37 μg/min) than males (GM 62.75 μg/min) (p=0.023) (Table 5.15).

5.5.1.2 Middle of the academic year

The significant difference was only noted on IgA secretion rate for the male-female comparison. Females still had statistically significantly lower secretion rate of IgA (GM, CI_{95}: 53.63 μg/min, 44.99-63.91 μg/min) than males (74.78 μg/min, 64.68 − 86.45 μg/min) (p=0.004) (Table 5.15).

5.5.1.3 End of the academic year

There was no significant difference on all the biomarkers levels for male-female comparison. The details of the results are shown in table 5.15
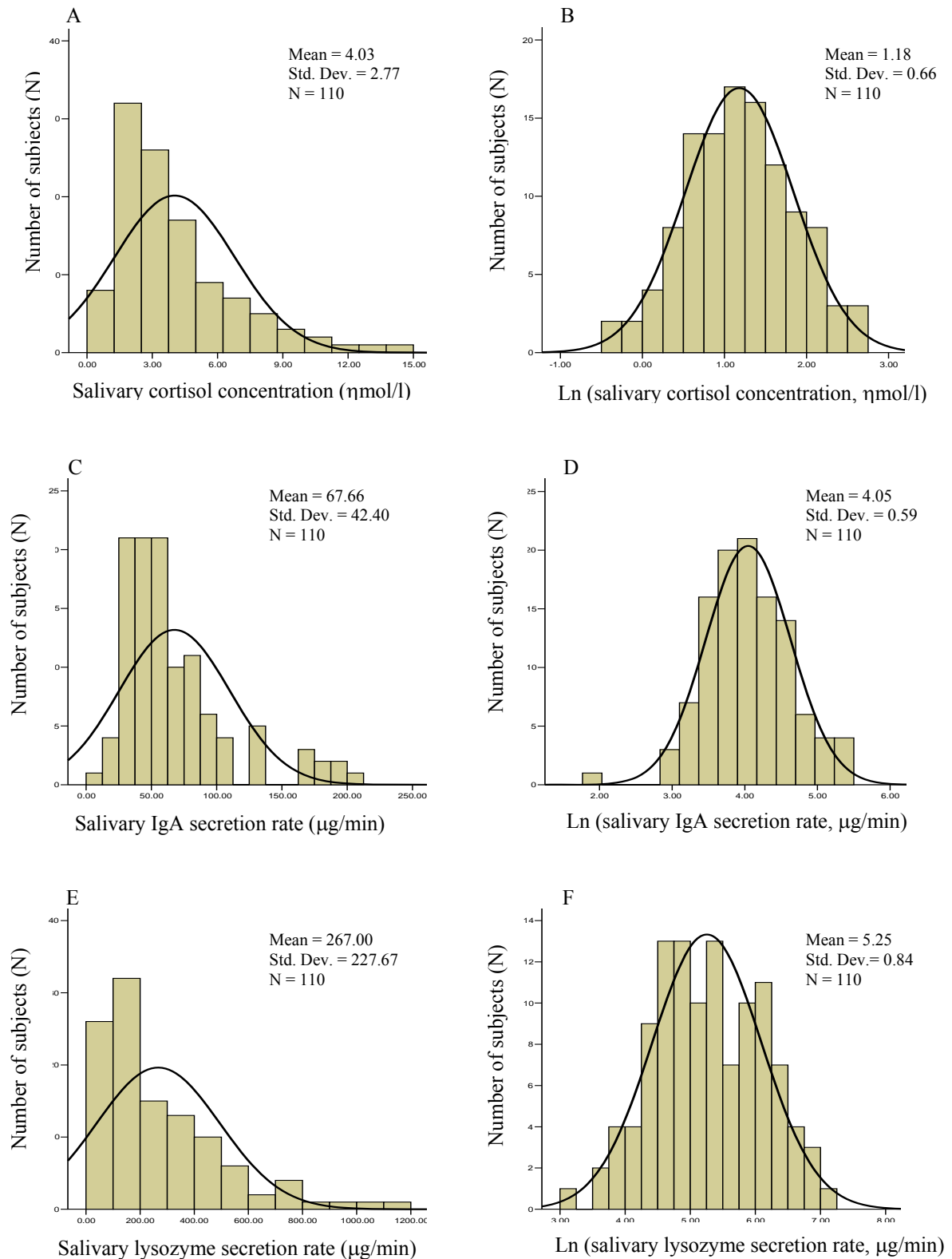
Figure 5.1 Salivary biomarkers at the **BEGINNING** of the academic year
(A) Untransformed frequency distribution of salivary cortisol concentration
(B) Natural logarithm transformed frequency distribution of salivary cortisol concentration
(C) Untransformed frequency distribution of salivary IgA secretion rate
(D) Natural logarithm transformed frequency distribution of salivary IgA secretion rate
(E) Untransformed frequency distribution of salivary lysozyme secretion rate
(F) Natural logarithm transformed frequency distribution of salivary lysozyme secretion rate

A
Mean = 5.82
Std. Dev. = 3.58
N = 96
Salivary cortisol concentration (ηmol/l)

B
Mean = 1.62
Std. Dev. = 0.50
N = 96
Ln (salivary cortisol concentration, ηmol/l)

C
Mean = 76.63
Std. Dev. = 41.24
N = 96
Salivary IgA secretion rate (μg/min)

D
Mean = 4.18
Std. Dev. = 0.57
N = 96
Ln (salivary IgA secretion rate, μg/min)

E
Mean = 153.68
Std. Dev. = 183.47
N = 96
Salivary lysozyme secretion rate (μg/min)

F
Mean = 4.53
Std. Dev. = 1.05
N = 96
Ln (salivary lysozyme secretion rate, μg/min)
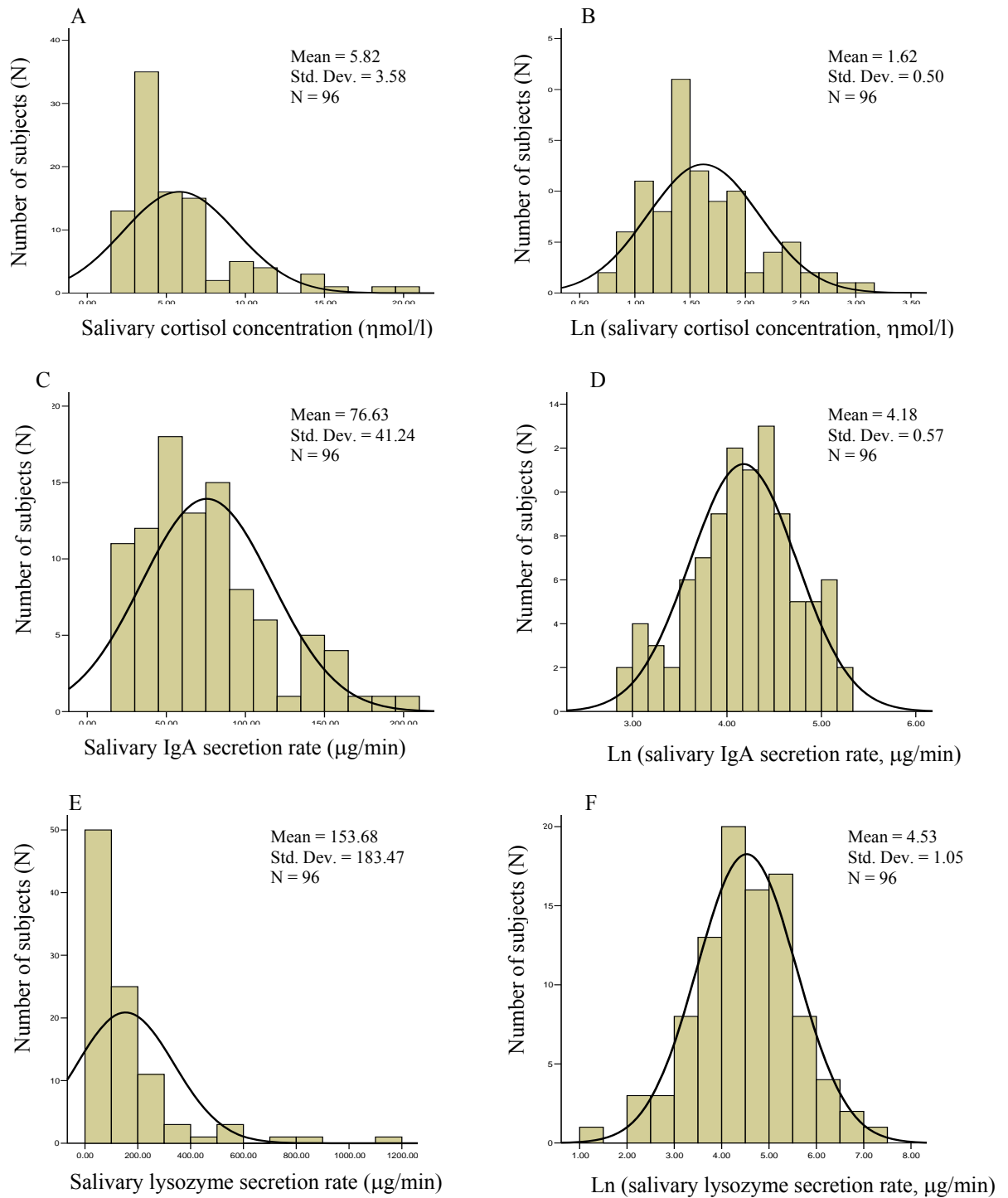
Figure 5-2 Salivary biomarkers at the **MIDDLE** of the academic year
 (A) Untransformed frequency distribution of salivary cortisol concentration
 (B) Natural logarithm transformed frequency distribution of salivary cortisol concentration
 (C) Untransformed frequency distribution of salivary IgA secretion rate
 (D) Natural logarithm transformed frequency distribution of salivary IgA secretion rate
 (E) Untransformed frequency distribution of salivary lysozyme secretion rate
 (F) Natural logarithm transformed frequency distribution of salivary lysozyme secretion rate

A — Mean = 4.94, Std. Dev. = 2.82, N = 106 — Salivary cortisol concentration (ηmol/l)

B — Mean = 1.45, Std. Dev. = 0.55, N = 106 — Ln (salivary cortisol concentration, ηmol/l)

C — Mean = 85.82, Std. Dev. = 40.13, N = 106 — Salivary IgA secretion rate (μg/min)

D — Mean = 4.35, Std. Dev. = 0.44, N = 106 — Ln (salivary IgA secretion rate, μg/min)

E — Mean = 277.91, Std. Dev. = 214.28, N = 106 — Salivary lysozyme secretion rate (μg/min)

F — Mean = 5.36, Std. Dev. = 0.74, N = 106 — Ln (salivary lysozyme secretion rate, μg/min)
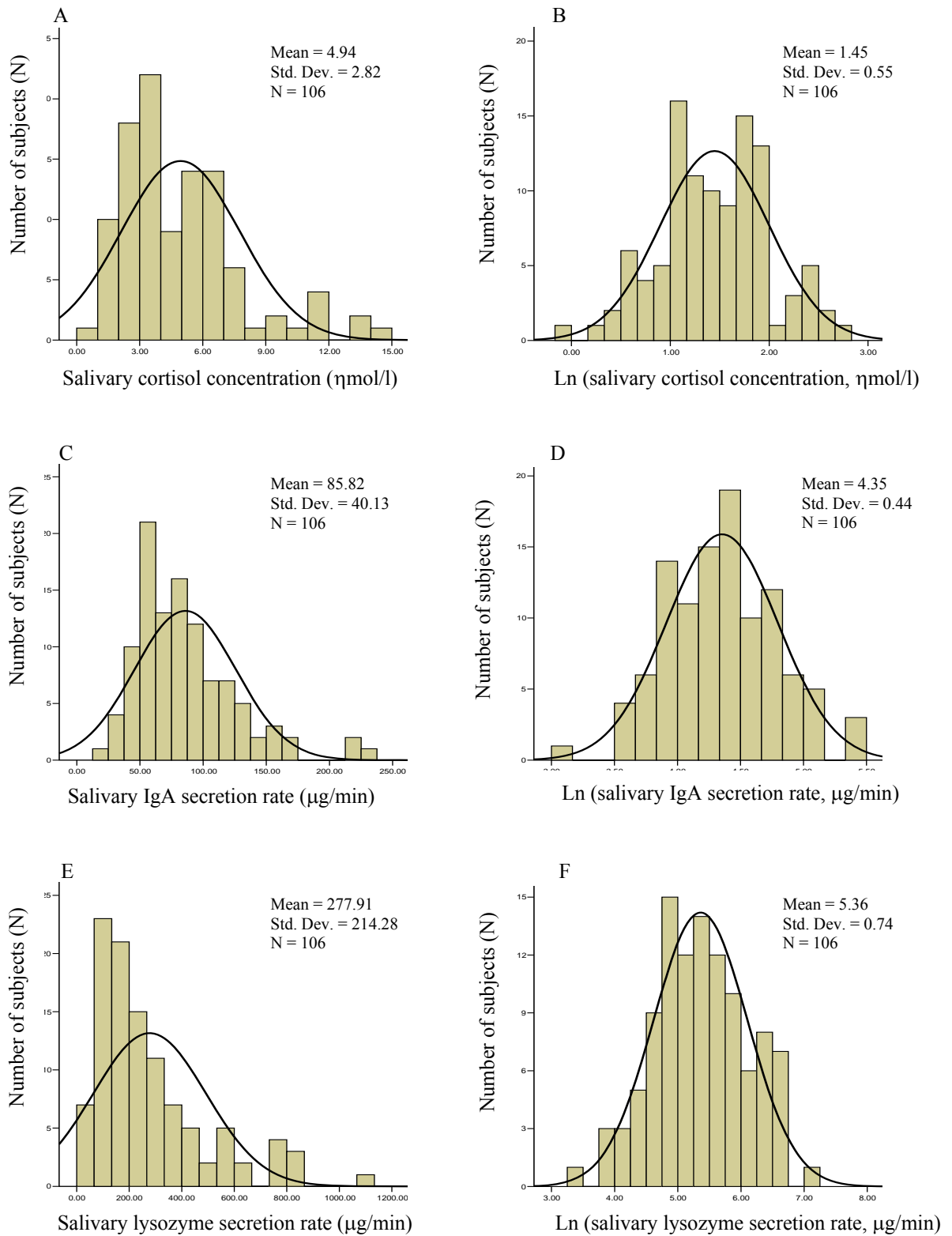
Figure 5-3 Salivary biomarkers at the **END** of the academic year
  (A) Untransformed frequency distribution of salivary cortisol concentration
  (B) Natural logarithm transformed frequency distribution of salivary cortisol concentration
  (C) Untransformed frequency distribution of salivary IgA secretion rate
  (D) Natural logarithm transformed frequency distribution of salivary IgA secretion rate
  (E) Untransformed frequency distribution of salivary lysozyme secretion rate
  (F) Natural logarithm transformed frequency distribution of salivary lysozyme secretion rate

Table 5.15. Geometric means (GM) and 95% confidence intervals (CI$_{95}$) for salivary biomarkers of males and female dental undergraduates at different times of the academic year (without URTI)

Biomarkers	Males GM CI$_{95}$			Females GM CI$_{95}$		
	Beginning (n=70)	Middle (n=52)	End (70)	Beginning (n=40)	Middle (n=40)	End (n=36)
Cortisol Concentration (ηmol/l)	3.39 (2.95, 3.94)	4.88 (4.39, 5.43)	4.42 (3.89, 5.01)	3.00 (2.38, 3.80)	5.29 (4.55, 6.16)	4.17 (3.57, 4.87)
IgA Secretion rate (μg/min)	62.75* (54.38, 72.41)	72.76[+] (64.43, 82.15)	81.23 (73.55, 89.72)	48.37* (41.17, 56.84)	54.11[+] (46.50, 62.97)	73.84 (64.95, 83.94)
Lysozyme Secretion rate (μg/min)	194.40 (159.60, 236.77)	111.09 (86.77, 142.21)	231.57 (195.74, 273.96)	185.97 (141.85, 243.84)	80.75 (59.91, 108.82)	193.00 (156.41, 238.17)

Independent-Samples T Test for male-female comparison
*At the beginning of the academic year, males had significantly higher IgA secretion rate than females (p=0.023)
[+]At the middle of the academic year, males had significantly higher IgA secretion rate than females (p=0.003)

5.5.2   Comparison on salivary cortisol, IgA and lysozyme at different times of the academic year for dental undergraduates from different academic classes.

5.5.2.1   Beginning of the academic year

Fourth year dental undergraduates had higher cortisol concentrations than those in 2nd year and 3rd year. The GM and CI_{95} of cortisol concentrations for the respectively academic classes were 4.78 ηmol/l (3.82 - 5.99 ηmol/l), 2.72 ηmol/l (2.12 - 3.48 ηmol/l) and 2.52 ηmol/l (1.97 - 3.22 ηmol/l). There were no statistically significant differences on sIgA and lysozyme secretion rates among all the four classes of dental undergraduates Table 5.16.

5.5.2.2   Middle of the academic year

The GM (CI_{95}) of sIgA secretion rates for the four academic classes were: 1st year =63.48 μg/min (53.25 -75.68 μg/min), 2nd year = 50.55 μg/min (39.89 -64.06 μg/min), 3rd year = 93.48 μg/min (75.58 -111.20 μg/min) and 4th year = 61.36 μg/min (45.86 -82.10 μg/min). Second year dental undergraduates had statistically significantly lower sIgA secretion rates than 3rd year dental undergraduates ($p < 0.05$). No significant differences among the four academic classes for the other two biomarkers (Table 5.17).

5.5.2.3   End of the academic year

It was noted that 1st year dental undergraduates had statistically significantly higher cortisol concentrations than 3rd and 4th year dental undergraduates. The GM (CI_{95}) of cortisol concentrations 1st to 4th academic classes were 5.37 ηmol/l (4.52 -6.37

ηmol/l), 5.13 ηmol/l (4.14 – 6.35 ηmol/l), 3.49 ηmol/l (2.95 – 4.13 ηmol/l) and 3.60

ηmol/l (2.79 – 4.64 ηmol/l), respectively. No statistically significant differences were

found for sIgA and lysozyme secretion rates among all the four academic classes of

dental undergraduates (Table 5.18).

Table 5.16. Geometric means (GM) and 95% confidence interval (CI$_{95}$) for salivary biomarkers of dental undergraduates from different academic classes at the beginning of the year (without URTI)

Biomarkers	Year 1 (n=28) GM CI$_{95}$	Year 2 (n=27) GM CI$_{95}$	Year 3 (n=28) GM CI$_{95}$	Year 4 (n=29) GM CI$_{95}$	Significant differences between classes
Cortisol					
Concentration (ηmol/l)	3.39 (2.66, 4.31)	2.72 (2.12, 3.48)	2.52 (1.97, 3.22)	4.78 (3.82, 5.99)	4 > 2, 3
IgA					
Secretion rate (μg/min)	46.87 (37.92, 57.93)	61.60 (48.91, 77.59)	54.87 (42.09, 71.51)	67.27 (56.08, 80.70)	NS
Lysozyme					
Secretion rate (μg/min)	190.83 (151.93, 239.70)	223.05 (155.23, 320.54)	214.37 (153.65, 299.08)	149.05 (104.72, 212.13)	NS

Significant difference between classes was calculated using general linear model to adjust for gender.
NS: Not significant

Table 5.17 Geometric means (GM) and 95% confidence interval (CI$_{95}$) for salivary biomarkers of dental undergraduates from different academic classes at the middle of the year (without URTI)

Biomarkers	Year 1 (n=25) GM CI$_{95}$	Year 2 (n=25) GM CI$_{95}$	Year 3 (n=23) GM CI$_{95}$	Year 4 (n=23) GM CI$_{95}$	Significant differences between classes
Cortisol					
Concentration (ηmol/l)	5.43 (4.33, 6.81)	4.80 (3.92, 5.88)	4.77 (3.80, 5.99)	5.26 (4.29, 6.45)	NS
IgA					
Secretion rate (μg/min)	63.48 (53.25, 75.68)	50.55 (39.89, 64.06)	93.48 (75.58, 111.20)	61.36 (45.86, 82.10)	3 > 2
Lysozyme					
Secretion rate (μg/min)	121.85 (89.07, 166.70)	85.72 (54.67, 134.41)	89.13 (58.35, 136.16)	78.10 (44.13, 138.21)	NS

Significant difference between classes was calculated using general linear model to adjust for gender.
NS: Not significant

Table 5.18. Geometric means (GM) and 95% confidence interval (CI$_{95}$) for salivary biomarkers of dental undergraduates from different academic classes at the end of the year (without URTI)

Biomarkers	Year 1 (n=26) GM CI$_{95}$	Year 2 (n=24) GM CI$_{95}$	Year 3 (n=27) GM CI$_{95}$	Year 4 (n=26) GM CI$_{95}$	Significant differences between classes
Cortisol					
Concentration (ηmol/l)	5.37 (4.52, 6.37)	5.13 (4.14, 6.35)	3.49 (2.95, 4.13)	3.60 (2.79, 4.64)	1 > 3, 4
IgA					
Secretion rate (μg/min)	71.87 (60.33, 85.60)	70.34 (57.34, 86.31)	84.82 (71.94, 99.99)	83.80 (71.00, 98.90)	NS
Lysozyme					
Secretion rate (μg/min)	288.13 (201.74, 411.50)	169.78 (123.82, 232.78)	248.37 (192.83, 319.90)	168.68 (135.25, 210.38)	NS

Significant difference between classes was calculated using general linear model to adjust for gender.
NS: Not significant

5.5.3 Correlation between stressors and perceived stress levels with salivary biomarkers

There was no statistically significant correlation for academic and non-academic stressors as well as the self-perceived stress levels with various salivary biomarkers at the beginning and the end of the academic year.

At the middle of the academic year, the clinical factor was negatively correlated with lysozyme secretion rates (Spearman's rho=-0.25, p=0.02). Total general life events score was noted to be negatively and statistically significantly correlated with sIgA secretion rates (Spearman's rho=-0.22, p=0.03). PSS score was positively correlated with cortisol (Spearman rho=0.33, p=0.00) but was negatively correlated with sIgA secretion rates (Spearman's rho=-0.20, p=0.05) (Table 5.19).

5.5.4 Correlation between modifying factors of stress with salivary biomarkers

There was no correlation between Type-A personality score with any of the salivary biomarkers. It was noted at the beginning of the academic year, less useful coping was negatively correlated with sIgA secretion rate (Spearman rho=-0.21, p=0.02). No statistically significant correlation on other coping strategies with salivary biomarkers was noted. Correlations between modifying factors of stress with salivary biomarkers are shown in Table 5.20.

Table 5.19. **Correlation between stressors, perceived stress levels with salivary biomarkers**

		Cortisol	IgA	Lysozyme
		Spearman rho (p)		
DES				
Academic Work	Beginning	-0.03 (0.77)	-0.14 (0.15)	-0.12 (0.22)
	Middle	0.10 (0.35)	-0.14 (0.17)	-0.07 (0.48)
	End	0.00 (0.97)	-0.11 (0.27)	-0.12 (0.23)
Faculty and Administration Factors	Beginning	0.05 (0.64)	-0.03, (0.76)	-0.16 (0.10)
	Middle	0.11 (0.30)	-0.06 (0.55)	-0.12 (0.26)
	End	-0.13 (0.17)	-0.11, (0.85)	-0.14 (0.14)
Clinical Factor	Beginning	-0.04 (0.70)	-0.15 (0.11)	-0.09 (0.34)
	Middle	0.06 (0.59)	-0.11 (0.30)	-0.25 (0.02)*
	End	-0.01 (0.94)	-0.11 (0.28)	0.00 (1.00)
Personal Factor	Beginning	0.01 (0.95)	-0.04 (0.66)	-0.13 (0.19)
	Middle	0.16 (0.13)	-0.02 (0.86)	-0.18 (0.08)
	End	0.00 (0.97)	-0.08 (0.44)	-0.13 (0.19)
General Life Events (total score)	Beginning	0.08 (0.43)	0.02 (0.81)	-0.05 (0.61)
	Middle	0.12 (0.25)	-0.22 (0.03)*	-0.11 (0.28)
	End	0.07 (0.51)	-0.03 (0.80)	0.07 (0.50)
Perceived stress scale score	Beginning	0.11 (0.27)	-0.10 (0.28)	-0.15 (0.12)
	Middle	0.33 (0.00)*	-0.20 (0.05)*	0.06 (0.57)
	End	0.02 (0.81)	-0.18 (0.06)	-0.17 (0.09)

* P<0.05

Table 5.20. **Correlation between salivary biomarkers with coping strategies and social support**

		Cortisol	IgA	Lysozyme
		Sperman's rho (p)		
Coping strategies				
Problem-focused coping	Beginning	-0.01 (0.93)	0.08 (0.42)	0.14 (0.14)
	End	0.05 (0.61)	0.06 (0.56)	0.03 (0.73)
Emotional-focused coping	Beginning	-0.16 (0.10)	-0.08 (0.41)	0.01 (0.93)
	End	0.10 (0.34)	-0.11 (0.26)	-0.16 (0.10)
Less-useful coping	Beginning	0.08 (0.42)	-0.21 (0.02)*	-0.16 (0.09)
	End	-0.04 (0.67)	-0.03 (0.75)	-0.06 (0.52)
Social support				
Total score	Beginning	-0.10 (0.28)	0.12 (0.21)	0.10 (0.29)
	End	0.04 (0.73)	0.11 (0.25)	0.07 (0.50)

* $p < 0.05$

5.6 Summary of results

In summary, this cohort of dental undergraduates perceived that academic work related matters was their prime stressor throughout the academic year. They had high scores on PSS especially at the middle of the academic year. Female dental undergraduates consistently had higher PSS scores than their male counterparts. It was found that there was an increased trend in the proportion of dental undergraduates who had total GHQ-28 scores of ≥ 5 from the beginning to the end of the academic year.

Approximately 75% of the dental undergraduates possess a type-A personality. There was a positive association between type-A personality with PSS and GHQ-28. This cohort of dental undergraduates frequently adopted problem-focused coping as their coping strategy. Female dental undergraduates more frequently used less-useful coping strategy than male dental undergraduates. Frequently adopted problem-focused coping was found to have an inverse relationship with PSS and GHQ-28. Conversely, less-useful coping was positively associated with PSS. More than three quarters of dental undergraduates sought support from their family members/friends and course-mates while approximately one third of them sought support from their supervisors or mentors. Total social support scores was negatively associated with PSS and GHQ scores.

Female dental undergraduates had lower IgA secretion rates than male dental undergraduates but not of cortisol concentrations or lysozyme secretion rates. Generally, most of the dental undergraduates had lower secretion rates of IgA and

lysozyme and higher concentration of cortisol at the middle of the academic year. At the beginning of the academic year, there was an inverse relationship between less-useful coping strategies and IgA secretion rates. At the middle of the academic year, it was noted that there was a positive correlation between PSS with cortisol concentration (Sperman's rho=0.33, p=0.00). In addition, weak inverse associations between PSS with IgA and lysozyme secretion rates were also noted. The Spearman's rho (p) for PSS and with IgA and lysozme secretion rates were -0.20 (0.05) and -0.18 (0.06), respectively. At the end of the academic year there were no correlations between stress levels and salivary biomarkers.

CHAPTER 6

DISCUSSION

This is the first longitudinal study to investigate the perceived sources and levels of stress experienced by dental undergraduates. It also looks into the moderating effect of personality, coping strategies and social support on their stress level. In addition, this study also investigates the relationship of salivary biomarkers with self-perceived stress levels.

6.1   Response rates and valid subjects

The present study received a very good response from the dental undergraduates throughout the study. All the dental undergraduates except one at the middle of the academic year and two at the end of the academic year, participated in the study and contributed salivary samples. The response rates ranged from 98.5% (end of the academic year) to 100% (beginning of the academic year). There was a 4-year longitudinal study conducted in Israeli dental students which also received a 100% response rate. However, that study was limited to only one class of 30 students (Peretz and Mann, 2000).

For salivary biomarkers analyses, only those subjects who satisfied our selection criteria were included. The exclusion criteria were flow rate < 1 ml/min or if the subject had an URTI during the saliva collection time or in the past one week. The majority of those who was excluded were due to URTI, which was beyond our

control. The valid subjects for salivary analyses were 82.1%, 71.6% and 79.1% for the beginning, middle and the end of the academic year, respectively. The valid subjects for this study were excellent as compared to other studies that had response rates of 27% (Ng et al., 1999) and 63.5% (Yang et al., 2002).

6.2 Socio-demographic characteristics

There were more males than females in this study. The gender ratio for all the classes was approximately 2:1. Dentistry is a male dominated profession (Rankin and Harris, 1990; Sugiura et al., 2005). With gender being one of the variables that could influence the stress levels, gender comparison analysis was carried out in this study.

The majority of the students were Chinese (92.5%) as the Chinese is the major ethnic group (76.8%) in Singapore (Census 2000 of Singapore). Most of them stayed either in their parent's house (77.6%) or a hostel (20.1%). A study indicated that living away from home posed certain stress to the students, but, their findings only involved their fourth year dental hygiene female students (n=8) (Al-Omari, 2005). However, Singapore is a small island with a total land area of 682.7 sq km; and local students staying in the hostel could still travel home with ease.

The dental undergraduates are aged between 19 to 25 years old; which is a very homogeneous age group. Therefore, age is not likely to be a confounding factor in this study.

It has been observed empirically by staff of Faculty of Dentistry that each class has its unique class dynamic and characteristics. Some classes are more cohesive, joyful, and less competitive that others.

6.3 Sources of stress and stress levels of dental undergraduates

Perceived sources of stress can be both academic and non-academic. This study was conducted to assess the sources of stress and stress levels of dental undergraduates throughout the academic year. Two different types of questionnaires were used in this study to assess the dental undergraduates' life stressors. The Dental Environmental Stress questionnaire (DES), which covered all possible stressors in the dental environment, was used to assess the academic stressors. The General Life Events (GLE) questionnaire assessed whether any significant life event had occurred to the students at any point of the academic year.

6.3.1 General life events (GLE) for dental undergraduates

The GLE was adopted from Cooper, Cooper and Cheng's Life Events Scale with deletion of those questions that were not related to our dental undergraduates. The scale used a 10-point Likert scale for each life event, based on it degree of upset or stress to the individual.

Total scores of > 50 points in GLE would indicate an individual experiencing stressful life events. This study showed that there was no significant difference between males and females as well as among the four academic classes throughout the academic year. Overall, there were only 6.7%, 1.5% and 1.5% of dental undergraduates who had scores of > 50 at the beginning, middle and the end of the academic year, respectively. It suggested that the general stressful life events contributed only a very small proportion to the dental undergraduates' stress level. It is an accepted phenomenon that academic factors contributes a significant amount of stress to students (Ko et al., 1999).

6.3.2   Dental Environment Stress (DES)

6.3.2.1   Beginning of the academic year

Many studies have reported that females perceived dental school to be more stressful than their male counterparts (Rosli et al., 2005; Sanders and Lushington, 1999; Westerman et al., 1993). This trend was not noticed in our Singapore dental undergraduates in this and an earlier (Yap et al., 1996) study. In this study, the overall mean score DES for male and female dental undergraduates were 2.47 and 2.53, respectively.

Compared to males, female dental undergraduates had significantly higher scores on two of the items on 'clinical factors', namely "atmosphere created by clinical faculty" and "expectation of dental school and what in reality it is like". This finding agreed

with other studies (Bradley et al., 1989; Garbee, 1981; Goldstein, 1979; Lloyd and Musser, 1985; Musser and Lloyd, 1985; Peretz et al., 1997) reported that female dental students were more stressed by clinical factors. Goldstein (1979) suggested that female students regard the faculty environment which is dominated by men as hostile and, therefore demonstrated higher stress scores. It must be noted, however, that the number of female students in most previous studies was low ranging from 16-26%. Interestingly, a study conducted in Malaysia revealed that their male dental students perceived that several items related to clinical training were more stressful to them. The proportion of female students in that study was 82% (n=268 of 325), leaving male students as the minority in the dental environment. It may be explained that being in the minority contributed to the feeling of discrimination in the clinical setting.

Male dental undergraduates perceived statistically significantly higher scores on "amount of cheating in dental school" than females. The score for males was 1.81 versus the score of 1.41 for females. The score on this item in contrast to other items for both the genders was fairly low. This finding may imply that male dental undergraduates are more concerned with regard to the academic integrity. The National University of Singapore is very strict with respect to student discipline; therefore "amount of cheating in dental school" was not a major stressor for the students.

Comparison on DES questionnaire items across four academic classes, 4th year dental undergraduates had significantly higher scores on the 12 items as showed in table 5.2.

Many of those items were clinical requirements related. Based on the combined mean stress scores, 4th year dental undergraduates experienced the highest perceived stress in the dental environment followed by 2nd year, 3rd year and 1st year students, and a statistically significant difference was noted between 4th year and 1st year students. It is not surprising that they felt that way as 4th year students experience increased pressure to fulfil graduation requirements in order to obtain the degree. This finding was in accordance with the American dental students study (Westerman et al., 1993), which reported that the 4th year was perceived to be the most stressful on DES questionnaire. In contrast, 1st year students may not have experienced stressors in their dental environmental yet at the beginning of the academic year. Thus, they were less stressful than others.

Second year dental undergraduates perceived 'clinical factors' as the most stressful to them even though they have not entered into the clinical year. It may reflect that the reality of the stressful nature of the dental school environment.

Overall, our dental undergraduates perceived 'academic work' was the prime stressor and "personal factors" as the least stressful event to them (Table 5.3). It may indicate that our dental students adapted well in handling their personal matters. Most of the students were from Singapore and neighbouring Malaysia, and their families and friends usually serve as the main source of support. In addition, the mentorship system offered by the faculty may also serve as another source of support for the students. In other studies (Heath et al., 1999; Sanders and Lushington, 1999; Westerman et al., 1993), financial resources have been indicated by students as one of

the stressful factors. However, this was not the case in Singapore students. In Singapore, our undergraduates are usually dependent on their family for financial support or obtain education loans from financial institutions throughout their study.

6.3.2.2   Middle of the academic year

At the middle of the academic year, there were intensive lectures, tutorials, seminars, practical, laboratory technique work, demonstrations and clinical treatment of patients. All students would definitely experience levels of stress. Therefore, it is not surprised that no differences between male and female dental undergraduates were found in term of overall mean of DES score, the four DES subscales as well as all the individual DES items.

There was also no difference in terms of overall mean DES score across the four academic years of students. This finding was in agreement with a study conducted in Tokyo Medical and Dental University, Japan, where they found no significant difference in the year of study for DES mean scores (Sugiura et al., 2005).

Findings from other studies (Humphris et al., 2002; Pau and Croucher, 2003; Peretz and Mann, 2000) showed that their students perceived highest stress from clinical factors during the transition year from pre-clinical to clinical training, because of the additional pressures on unfamiliar patients' care. In this study, at this point of time, it was noted that 2nd year scored statistically significantly higher on "academic work" and "clinical factor" than 1st year dental undergraduates (Table 5.3). It may be due to

the 2nd year dental undergraduates had started their practical classes related to dentistry; hence creating the awareness of the difficulties in clinical training and elevating the perception of stress. It caused them to be particularly stressed regarding the 'academic work' and 'clinical factors'.

When comparison was made in all the DES items among the undergraduates from different academic years, 4th year students perceived more stress on items such as "atmosphere created by clinical faculty", "expectations of dental school and what in reality it is like" and "lack of input into the decision-making process of the school". It may be attributed to the fact that during the clinical years, they have to spend most of their time at the Faculty of Dentistry clinics treating patients under the supervision of staff, thus they felt more intense of the stress imposed by the faculty staff. It was also reflected in the subscale of faculty and administrator factors (FF) that 3rd and 4th year dental undergraduates had significant higher scores that 1st year dental undergraduates statistically (Table 5.5). Fourth year undergraduates also felt financial responsibilities imposed as a significant amount of stress (score: 2.94). This could be because they were be graduating soon and would no longer be dependent on their parents for financial support.

6.3.2.3   End of the academic year

It was noted that at the end of the academic year, both male and female dental undergraduates perceived 'atmosphere created by clinical faculty' was the top stressor to them. Special attention should be drawn to this finding. This may reflect a problem with regard to approachability of dental staff of the whole faculty. Male

students were more stressed than females on 'rules and regulations of the school'. This may reflect the dominant characteristics of males, who are dislike being controlled and abided by rules and regulations.

At this point of time, 2nd year dental undergraduates perceived more stress on many of the DES items than dental undergraduates in other academic years. They scored the highest on overall DES than other years, although the difference was statistically significant from 1st year dental undergraduates (Table 5.6). This may be due to the transition period from preclinicals to clinicals for 2nd year students. They may encounter difficulty in learning clinical procedures and making clinical decisions related to dentistry; hence this would increase their perception of stress in the dental environment. The similar finding was noted in Malaysian dental undergraduates where their 2nd year students reported high stress levels, resulting in some of them even considered switching courses to some entirely different fields of study (Rosli et al., 2005).

Generally, throughout the academic year, the top stressors for all the dental undergraduates were 'academic work' related. To become a responsible dental professional, students have to reach high levels of knowledge and professional skills, as well as develop good attitudes towards patients care, all within a short period of time. Thus, it is difficult to eliminate all stress-related problems in a dental education programme.

6.3.3 Perceived stress scale (PSS)

The PSS is a scale to measure the degree to which situations in a person's life are appraised as stressful. Items were designed to tap how respondents find situations in their lives unpredictable, uncontrollable or overloaded. These three issues repeatedly have been found to be central components of the experiences of stress. The scale also includes a number of direct queries about current levels of experienced stress. The PSS was designed for use in community samples with at least a junior high school education. The items are easy to understand, and the response alternatives are simple to grasp. Moreover, the questions are of a general nature and hence are relatively free of content specific to any subpopulation group. (Cohen and William, 1988).

6.3.3.1 Comparison of self-perceived stress levels between genders and among dental undergraduates at different times of the academic year

Females dental undergraduates had statistically significantly higher PSS-10 score than males at the beginning and middle of the academic year (results in Table 5.9) though no significant difference of mean DES and GLE scores were found between them during those periods of time. Many reports have suggested that females perceived the dental school to be more stressful than their male counterparts (Naidu et al., 2002; Pau and Croucher, 2003; Rajab, 2001; Sanders and Lushington, 1999; Sugiura et al., 2005; Westerman et al., 1993). It was suggested that gender differences in self-reported questionnaires, although significant, must always be taken with some caution, because, due to societal norms and conventions, males may tend to not fully expose

their feeling, while females were more willing to express their anxieties (Peretz & Mann, 2000)

The overall mean scores of PSS from the beginning to the end of the academic year were 19.89, 20.79 and 20.35, respectively. These scores were relatively higher than that reported in a population norm of 13.03 (Cohen and William, 1988). It is also higher as compared to the study conducted by Pau and Croucher, (2003) on students from Barts and The London, Queen Mary's School of Medicine and Dentistry, who had a mean PSS of 17.73 (Pau and Croucher, 2003). This may be attributed to the fact that Singapore is a highly competitive society where academic excellence is highly regarded (Yap et al., 1996). Moreover, in Singapore there is only one dental school, with limited available places. Thus, there are high expectations from family and society for their success which leads to higher level of stress. In Humphris et al's study, it was found that the level of emotional fatigue was higher in dental undergraduates when compared to medical undergraduates (Humphris et al., 2002). This is in agreement with Garbee's findings that dental students experienced more stress than the average university students (Garbee, 1981)

There were no significant differences of overall PSS levels from beginning to the end of the academic year for all the students. However, our dental undergraduates generally scored the highest on PSS at the middle of the academic year. This may be due to the intensively on-going of various classes during that period. Thus, it was the most stressful period for the students. Overall, 4th year dental undergraduates scored

the highest in PSS as compared with other classes. It is understandable that they would experience increased pressure in order to fulfil the graduation requirements.

6.3.4   General Health Questionnaire (GHQ-28)

The GHQ is mainly focused on breaks in one's normal functioning rather than on life-long traits; therefore it only covers personality disorders or pattern of adjustment where associated with distress. It is not intended to detect severe illness such as schizophrenia or psychotic depression (McDowell and Newell, 1996).

6.3.4.1   Comparison general mental health between genders and among dental undergraduates at different times of the academic year

Generally, female dental undergraduates had higher GHQ-28 scores than their male counterparts (Table 5.10). A study on dental undergraduates from Newcastle University also reported that 72% (n=36) of their final year students scored >4 in the GHQ-30, with females (87.5%, n=21) having a significantly higher score than their male counterparts (57.7%, n=15) (Newbury-Birch et al., 2002). Higher scores for females may be attributed to the fact that females are more expressive about their feelings.

The overall levels of caseness on the GHQ-28 in this cohort were 46.3% at the beginning, 56.4% at the middle and 63.6% at the end of academic year. These figures showed an increased trend in psychological morbidity. These figures were also

markedly higher than those reported in other studies such as those on Scottish ambulance personnel (32% of caseness) and health service consultants (21% of caseness) in Scotland (Alexander and Klein, 2001). However, there might be high false positive results in using GHQ scale. In a study conducted by Weinberg and Creed, 2000 (Weinberg and Creed, 2000) using GHQ-12 and setting the cut-off of >4 as a case, it found that more than half of the 132 respondents who had scored >4 were not caseness at clinical interview (Weinberg and Creed, 2000). Based on the Whitehall II study of 10,314 London-based civil servants men and women between 35 and 55 years, Stansfeld and Marmot suggested that people in lower employment grades tend to under-report minor psychiatric disorders on the GHQ relative to those in higher employment grades. Therefore, they advised to treat the finding based on questionnaire with caution (Stansfeld and Marmot, 1992).

Professional training is regarded as highly stressful for most of the students. The results of GHQ in this study indicated that a high proportion of our dental undergraduates suffered certain degrees of psychological distress. This study results were in line with other studies on undergraduates. The study in the Europe on 331 dental undergraduates also reported that over a third of the students (36%) had significant psychological distress (Humphris et al., 2002). In a study of comparing law and medical undergraduates in the National University of Singapore which used GHQ-28 to identify the psycho-emotional disturbances, it was reported that high proportions of medical (57%) and law students (43%), were considered at risk for minor psychiatric morbidity. However, due to the cramped curriculum and heavy academic load of the students, they were unable to proceed to further assess for possible psychological disorders among each individual high scorer (Ko et al., 1999).

6.3.5 Relationships between perceived stressors, stress levels and mental health

The DES scores were positively and significantly correlated with GHQ at three different time points of the academic year (r= 0.49, p=0.00). The positive relationships between perceived stressors, and mental health throughout the year demonstrated that the there is a causal link between perceived dental environment stress and psychological well being. Ample studies report that undergraduates experience a tremendous amount of stress. The curricula in tertiary education have always come under strong criticism for being grossly overloaded with information. The stressful environment can often exert a negative effect on the academic performance, physical health and psychological well-being of the undergraduates. If it is not managed properly, these adverse effects can be a waste of valuable human resource. (Ko et al., 1999)

6.4   Modifying factors of stress

6.4.1   Type A personality

Most of the dental undergraduates possess a strong type-A personality. It was also noted in our study that those who have stronger Type A behaviour, reported higher levels of mental distress ($r = 0.20$, $p=0.02$). Research seems to suggest that certain personality types predominant among individuals attracted to the dental profession (Mozer et al., 1990). In the study of 300 dental students from the University of North Carolina School of Dentistry, George et al (George et al., 1987) found that Type A behaviour was strongly associated with higher stress levels. Type As have been shown to have greater physiological arousal under a variety of stressful conditions. One can readily imagine how hard-driving behaviour, competitiveness, impatience, time urgency, and high task involvement could interact with the demands of dental education to produce a more stressful environment. Another study has demonstrated that Type A persons report more work stress and job tension (Kittel et al., 1983).

6.4.2   Coping strategies

Generally, there was no change in the coping patterns for this cohort of dental undergraduates from the beginning to the end of the academic year. The study findings were in agreement with others that females were more likely than men to cope with emotion-oriented behaviours (Tamres et al., 2002). Overall, our dental

undergraduates adopted problem-focused coping when they were faced with stressful situation.

Age has been shown to affect coping strategies (Diehl et al., 1996), however, our dental undergraduates students were within the narrow age band of 19-23 years, therefore, it is not surprising to note that there was no difference in coping strategies across the four academic years.

As problem-focused and emotional-focused responses are considered more adaptive than less-useful coping, adoption of positive and adaptive coping strategies were associated with lower perception of stress and elevated mental health (results in Table 5.14).

6.4.3 Social support

In this study, no difference was found in term of various types of personnel the dental undergraduates could rely on between the beginning and the end of the academic year. Generally, they sought support from family/friends and course mates when they were in need. Another study on Singapore medical and law students (Ko et al., 1999) found that more than three quarters of the surveyed students turned to their friends and classmates for assistance when they had problem. It is very important to have peer-to-peer interaction in venues such as tutoring programs, support group, and other student-led and managed activities.

A system of mentoring fresh undergraduates by older students may provide the former with invaluable support. However, only a small proportion of our dental undergraduates sought support from their mentors. The positive responses ranged from 35.6% (beginning) to 37.3% (end) during the academic year. Therefore, there is a need to improve this system to make it more effective.

It was noted that at the beginning of the academic year, more females (47.1%) turned to religion as a source of support than males. The reason for this observation is unexplainable. Nevertheless, by and large about one in three students turn to religion for help as reported in the study on stress and psychological well-being of medical and law students in Singapore. Ko et al further suggested that in a pragmatic and materialistic society like Singapore, it is important that religion continues to play an important role in providing counsel and positively influence the value systems of the youths, in order to produce professionals not just with a good brain, but also with a good heart (Ko et al., 1999).

On the whole, our dental undergraduates had some kind of support when they were in need. The results also indicated that social support was negatively associated with GHQ and PSS at the beginning and at the end of the academic year. In general, this study findings support the hypothesis that social support can act as a buffer against the adverse effects of stress on students' mental health (Bovier et al., 2004).

6.5 Salivary biomarkers

6.5.1 Salivary IgA

Psychoneuroimmunological research has shown that psychosocial factors, including stress, social support and emotion may affect susceptibility to infectious diseases by influencing the immune system (Jemmott et al., 1983; Kiecolt-Glaser et al., 1996). Secretory Ig A (sIgA) plays an important in the defence mechanism of mucosal immunity; it has been chosen as a measure of resistance to infectious disease. This immunological stress marker has increasingly been chosen as an objective measure to complement the subjective questionnaire method.

6.5.1.1 Comparison on levels of salivary IgA between male and females dental undergraduates

In this study, salivary IgA secretion rates were lower in female dental undergraduates at the beginning and middle of the academic year. However, a study had shown that gender was not associated with salivary IgA (Kugler et al., 1992). The explanation for the lower IgA secretion rates could be females experienced more stress psychologically. From the results obtained through the PSS, that female dental undergraduates were more stressed than male counterparts. There was no significant difference of salivary IgA rates between the both genders at the end of the academic year. It maybe because that was the tail end of the course and most of them were in a relaxed mood.

6.5.1.2 Comparison on levels of salivary IgA among dental undergraduates from different academic classes

When comparison on IgA secretion rates among the dental undergraduates from different academic years, the difference only noted at the middle of the academic year. At the middle of the year, 2nd year dental undergraduates had significantly lower sIgA secretion rate than 3rd year dental students. It was reported that stress among students was particularly high during the first two years of dental undergraduates (Newton et al., 1994). This finding was in agreement with the Malaysia study that their preclinical students also reported high level of stress (Rosli et al., 2005). It is understandable that 2nd year dental undergraduates were experiencing the transition period from a totally non-clinical to clinical training, that they found it difficult in learning clinical procedures. Thus, it would impose a high psychological stress to them.

6.5.1.3 Relationship between salivary IgA with stress and modifying factors of stress

Based on the subjective (questionnaire) findings, it showed that the middle of the academic year was the most stressful period for the dental undergraduates. At this time point, salivary IgA secretion rate was significantly and inversely correlated with the self-perceived stress level (PSS-10). It was also significantly and negatively correlated with total score of general life events (GLE). These results confirmed findings of previous studies on various group of nurses where those who reported

higher levels of psychological stress had significantly lower levels of salivary IgA (Ng et al., 1999; Yang et al., 2002). IgA is produced locally by plasma cells in the salivary gland. Chronic psychological stress may decrease salivary IgA secretion (Hucklebridge et al., 2000) by reducing recruitment of precursors of plasma cells in the salivary gland or by directly affecting the immunoglobulin producing activity of the plasma cell (Tsujita and Morimoto, 1999).

In this study, it was found that those who adopted less-useful coping or avoidance coping also had lower lgA secretion rate. A study was conducted to investigate whether active and avoidance coping methods were differentially related to immune function depending on stress levels. Concanavalin A (Con A) was used as a stimulant for T-cells proliferation in the study. In comparison to individuals subjected to high stress level, lower stress level individuals who adopted avoidance coping had a higher T cells proliferation profile, when stimulated by Con A. This may suggest that our dental undergraduates who adopted avoidance coping and had lower IgA secretion rate were experiencing high level of stress. It has been shown that avoidance coping was associated with poorer immune functions (Goodkin et al., 1992b). Findings in this study may indirectly support the evidence of the moderating effects of positive coping on stress-immune relationship.

6.5.2   Salivary Lysozyme

At the three different time points of this study, lysozyme secretion rates for females were generally lower than males, even though females had perceived higher level of

stress. However, the differences in lysozyme secretion rates were not statistically significant. Salivary lysozyme secretion rate were found to have a moderate positive relationship with IgA secretion rates (Pearson's r = 0.4; p<0.01). There was also no significant negative relationships between salivary lysozyme secretion with any stressors or self perceived stress levels (except for subscale of DES on clinical factor), whereas the other studies indicated have otherwise (Perera et al., 1997; Yang et al., 2002). Therefore, further investigations using salivary lysozyme as a stress biomarker are needed before any conclusion can be drawn

6.5.3   Salivary cortisol

Cortisol is a major glucocorticoid in humans that reflects adrenocortical activity. Activation of the hypothalamus-pituitary-adrenal (HPA) axis and the subsequent release of cortisol are major components of the physiological stress responses. Salivary cortisol accurately reflects serum free cortisol, the major physiological active component (Kirschbaum and Hellhammer, 1994). Under basal conditions, cortisol secretory activity is characterised by peak level following awakening accounting for a morning acrophase. Thereafter it falls with a declining trend over the remainder of the day. (Edwards et al., 2001).

In this study saliva samples were collected approximately from 11 a.m to 1 p.m which was presumed to be 3 hours after the participants' awakening times. This timing was chosen as to avoid contamination of cortisol results due to morning acrophase in early

morning hours and due to external stimulation such as meal-related cortisol secretions.

6.5.3.1 Comparison on levels of salivary cortisol between male and females dental undergraduates

In this study, no significant difference of cortisol levels was found between male and female dental undergraduates at three different time points throughout the academic year. However, the study by Steptoe et al on socioeconomic status (SES) and stress-related biological responses found that in males, cortisol output over the day was greater in participants of lower SES. But in females, the reverse was true, with more cortisol in the higher SES group. It indicated that there was a sex difference in SES and cortisol (Steptoe et al., 2003). Nonetheless, such observation was not noted in this study. The non–significant difference of cortisol levels between male and female dental undergraduates observed in this study might be due to the following reasons: firstly the participants' SES was not taken into consideration as a variable. Secondly, in this study, generally female dental undergraduates were more religious than males as higher percentage of them turn to religion group for help when they were in need. A previous study suggested that spiritual and /or religious individuals may experience a protective effect against the neuroendocirne consequences of stress. In this study, participants with high composite religiosity/spirituality scores, levels of forgiveness and frequency of prayer showed lower cortisol response (Tartaro et al., 2005). Thirdly, there is a wide variation of cortisol diurnal patterns between individuals. A study on individual differences in the diurnal cycle of cortisol found that 17% of the

109 studied participants did not exhibit diurnal cycles of cortisol even though they did not differ from those with normal cycle on demographic factors, baseline psychological measures, health behaviour, and daily experiences during the assessment period (Smyth et al., 1997).

6.5.3.2   Comparison on levels of salivary cortisol among dental undergraduates from different academic classes

When comparing cortisol levels among different academic classes, it was noted that 4th year dental undergraduates had significantly higher cortisol level than 2nd and 3rd year dental undergraduates at the beginning of the academic year. While at the end of the academic year, 1st year dental undergraduates had significantly higher cortisol level than 3rd and 4th year dental undergraduates. However, based on their self-perceived stress levels (PSS), this trend was not observed. It showed that there were differences between subjective stress outcomes and objective cortisol responses. It is not surprising to observe the discrepancy between the objective and subjective outcomes. According to Feldman et al, it was a typical finding in psychobiological research, since physiological responses and affective changes are only loosely coupled (Feldman et al., 1999). Kunz-Ebrecht et al further suggested that subjective rating cannot be employed as proxies for biological responses, and that objective measures are needed to document associations between psychosocial and biological outcomes (Kunz-Ebrecht et al., 2004). Nevertheless, at the middle of the academic year, cortisol levels were moderately and positively correlated with PSS scores. It

reflects that at the middle of the academic year that was the most stressful period experienced by the students.

By interpreting the dental undergraduates stress levels based on the cortisol output, it is understandable that 4th year dental undergraduates felt the most stressful at the beginning of the academic year. It can be cross referenced based on their self-perceived dental environmental stressors. At the beginning of the academic year, 4th year dental undergraduates had higher scores on many (n=13) of the DES items than the others. The final year of the study is a critical year for most of the students. Moreover, Singapore is a highly competitive society where pride and excellence have been inculcated at both family and national level and failure is often met with disapproval (Yap et al., 1996). Thus, students might anticipate the stress of fulfilling the graduation requirements. The study in India indicated that fear of facing parents after failure was the most stressful item for their dental students of all years (Acharya, 2003). Whereas, only final-year Malaysia dental undergraduates perceived this item more stressful compared to students in the other academic years (Rosli et al., 2005). Studies had reported increased cortisol levels during anticipation of stressful situations such as cardiac surgery, dental treatment procedures, oral presentation as well as written examination (Czeisler et al., 1976; Lacey et al., 2000; Miller et al., 1995; Ng et al., 2003a). A significant positive relationship between cortisol level and workload stress was also found in the study of 158 male air traffic controllers (Zeier et al., 1996). Therefore, the findings in this study support the hypothesis that current problems and anticipation of stressful events can elevate the salivary cortisol levels.

In the middle of the academic year, all the students were stressed by the intensive course work and, it was noticed that there was no difference on the cortisol levels among all the four academic years of dental undergraduates. Nevertheless, generally the cortisol levels for all the students were higher at this time point as compared to the beginning and the end of the academic year (Table 5.16 to Table 5.18). It showed that the middle of the academic was the most stressful period for the dental undergraduates.

At the end of the academic year, 1st year dental undergraduates had significantly higher cortisol level than 3rd and 4th year dental undergraduates. This may be explained by the fact that the end of the 1st year and start of the 2nd is associated with the beginning of exposure to the clinical dental environment

6.5.3.3   Relationship of salivary cortisol and modifying factors of stress

In this study, no association was found between coping strategies and social support with salivary cortisol levels. This may be attributed to the fact that the questionnaire asked about events which happened in the past two months, while, cortisol has a very short half-life time of less that one hour (Kirschbaum and Hellhammer, 1994). A single salivary cortisol measurement might not show a relationship with the modifying factors that occurred in the past two months.

6.6 Limitation of the study

There were some limitations of this study which might affect the interpretation of the results.

6.6.1 Study design

One limitation of the study was its small sample size, which reduced the power analysis for further stratification analyses. However, the number of dental undergraduates (n=134) participated in the study represent almost 100% of the response rate, which is the whole population of the dental undergraduates in Singapore in that particular academic year, and in fact was the strength of the study.

6.6.2 Study Instruments

Although standard questionnaires were used to assess the dental undergraduates' stressors and stress levels, the self-reported nature of the data would inevitably suffer from voluntary and involuntary bias. The bias may include recall bias, exaggerating or under-reporting of the actual situation for fear that others might know of their real situation. Moreover, the questionnaires were developed and validated in the West; those questionnaires might not be the ideal instrument to accurately assess stress the in Asians. However, to our knowledge no such questionnaires have been specifically designed or tested for measuring dental environmental stress for the Asian countries.

Nonetheless, our dental undergraduates were proficient in English and had no problems in understanding the questionnaires.

6.6.3   Statistical analyses

The responses for the questionnaires were made on the ordinal scales, non-parametric techniques should have been employed to analyse the data. However, most of the studies in the existing literature used parametric methods to analyse the data from questionnaires such as DES, PSS and COPE. In order to ease the comparison with other studies, parametric methods were used to analyse some of the ordinal data of my questionnaires as well. In addition, the distributions of the data on DES and PSS and COPE were checked for their normality. Results from a test of skewness of these data were <+1 or -1. This suggested that the data were generally normally distributed.

Recoding of GHQ and GLE data into dichotomous variables may result in loss of some information and that the threshold value for dichotomizing GHQ and GLE data may not be absolutely applicable in the local/Asian context. However, the recoding of the questionnaire was performed according to the instructions of the standard original questionnaires. In addition, comparisons of the findings were made with the studies conducted either locally or internationally that employed that same recoding method.

6.6.4   Saliva sample collection

Salivary biomarkers were included in this study as to complement the subjective assessment of stress. However, in order not to disturb the students' academic schedule, only a single point collection of saliva sample for all three biomarkers analyses was carried out. This may affect the results in this study especially salivary cortisol analysis. Cortisol shows a pronounced variation across the time of the day, and it has been argued that stress may alter the pattern (Dahlgren et al., 2005). Studies showed that stress and increased workload often elevate cortisol levels, particularly in the morning (De Vente et al., 2003; Kunz-Ebrecht et al., 2004; Steptoe et al., 2000). In addition, it was suggested that the saliva sample collection for cortisol analysis in relation chronic psychological stress should be done at awakening time rather than clock time so as to increase the sensitivity and reliability of cortisol measurement (Clow et al., 2004). In this study, this criterion was not complied with.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The cohort of dental undergraduates in this study, regardless of academic class and gender, perceived their main source of stress as academic related matters throughout the academic year. They perceived high level of stress throughout the academic year especially at the middle of the academic year. The difference found on source of stress between male and female dental undergraduates was negligible, although female dental undergraduates showed consistently higher self-perceived stress levels than their male counterparts.

There was an increased trend in psychiatric morbidity which was measured using GHQ-28 from the beginning to the end of the academic year for this cohort of dental undergraduates. These findings support previous results that dentistry training is stressful. It was also noted that females were more expressive about their feeling.

The majority of the dental undergraduates possess a Type-A personality. There was a positive association between Type-A personality with PSS and GHQ. This finding supported the hypothesis that dental school, being a stressful environment, could provide conditions that elicit the display of Type-A behaviour.

Generally, dental undergraduates frequently adopt problem-focused coping as their coping strategy. Problem-focused coping was found to have an inverse relationship with PSS and GHQ. In contrast, those who frequently adopted less-useful coping strategies had higher levels of PSS. This finding supports the moderating effect of positive coping on one's stress level and mental health.

One the whole, a higher percentage of dental undergraduates sought support from their family members/friends and course-mates as compared to their mentor or supervisors. Those who indicated that they had more social support, also reported to have lower self-perceived stress levels and psychiatric morbidity. Therefore, it is concluded that social support has a buffering effect against stress and has positive influence on one's mental health.

The salivary biomarkers were better correlated with PSS at the middle of the academic year. It was also noted that at the middle of the academic year, dental undergraduates had lower secretion rates of salivary IgA and lysozme and higher concentration of salivary cortisol. This indicated that the middle of the academic year was the most stressful period for dental undergraduates. At that time point, it was found that there was a significant positive correlation between PSS and cortisol concentration. In addition, inverse associations between PSS with IgA and lysozyme secretion rates were also noted. This could be concluded that salivary biomarkers of stress correlate better at higher level of stress. The findings supported the hypothesis that stress activates HPA axis and suppresses immunity.

7.2   Recommendations

Based on the findings of this study, the following recommendations are made for (i) improvement on the validity of future studies on dental environmental stress and (ii) management of dental undergraduates stress.

7.2.1   Choosing appropriate time for assessing dental environmental stress

The findings of this study showed that the middle of the academic year was the most stressful period for dental undergraduates. Both subjective and objective measurements had a better agreement at this time point of the study. Therefore, to conduct any stress study especially for cross-sectional studies; it is important to identify the actual stressful situation to be the appropriate period of testing.

7.2.2   Stress management program for dental undergraduates

The Behaviour Science module entitled "Stress in dentistry" which consists of six one-hour lectures covers topics like stress in the dental profession, stress coping & management strategies, is offered to dental undergraduates at year 3. However, most of the dental undergraduates reported they had high level of stress in all 4 classes. The FoD might to consider offering some other "stress management programs" to help their undergraduates to manage their stress better in their dental training.

7.2.3 Future study using salivary biomarkers for assessing stress

In this study, cortisol levels were found to be moderately associated with the dental undergraduates self-perceived stress level at the middle of the academic (the most stressful period). It has been argued that chronic stress may alter the cortisol diurnal pattern. While increased cortisol secretion is considered to be an adaptive mechanism when stress, chronically elevated cortisol level may be harmful. It is less clear what the effects of chronic stress of dental environment on cortisol diurnal profile. Therefore, future studies on dental environmental stress may be needed to consider assessing cortisol diurnal profile in relation to the stress levels.

References

Acharya, S. (2003). Factors affecting stress among Indian dental students. J Dent Educ *67*, 1140-1148.

Alexander, D. A., and Klein, S. (2001). Ambulance personnel and critical incidents: impact of accident and emergency work on mental health and emotional well-being. Br J Psychiatry *178*, 76-81.

Allen, R. (1983). Chapter 4. The stress prone-personality. In Human Stress Its Nature and Control (New York, Macmillan Publishing Company), pp. 40-53.

Al-Omari, W. M. (2005). Perceived sources of stress within a dental educational environment. J Contemp Dent Pract *6*, 64-74.

Arnetz, B. B., Edgren, B., Levi, L., and Otto, U. (1985). Behavioural and endocrine reactions in boys scoring high on Sennton neurotic scale viewing an exciting and partly violent movie and the importance of social support. Soc Sci Med *20*, 731-736.

Bailey, D. (1987). Understanding stress, Vol Part One (London, H.M.S.O.).

Benjakul, P., and Cheunarrom, C. (2000). Student adjustment problems in two dental schools in Thailand. J Dent Educ *64*, 365-369.

Billings, A. G., and Moos, R. H. (1981). The role of coping responses and social resources in attenuating the stress of life events. J Behav Med *4*, 139-157.

Blonnna, R. (1996). Part 2. Mental and Emotional Basis of Stress. In Coping with Stress in a Changing World (St. Louis, Mo., Mosby-Year Book, Inc.), pp. 37-40.

Boscarino, J. A. (1996). "Posttraumatic stress disorder, exposure to combat, and lower plasma cortisol among Vietnam veterans: findings and clinical implications." J Consult Clin Psychol **64**(1): 191-201.

Bovier, P. A., Chamot, E., and Perneger, T. V. (2004). Perceived stress, internal resources, and social support as determinants of mental health among young adults. Qual Life Res *13*, 161-170.

Bradley, I. F., Clark, D. C., Eisner, J. E., De Gruchy, K., Singer, D. L., Hinkleman, K., Gelskey, S. G., and Wood, W. W. (1989). The student survey of problems in the academic environment in Canadian dental faculties. J Dent Educ *53*, 126-131.

Brown, J. C., and Barnett, J. M. (1983). Faculty response to personal problems of students: a survey of dental educators. J Am Coll Dent *50*, 23-28.

Burk, D. T., and Bender, D. J. (2005). Use and perceived effectiveness of student support services in a first-year dental student population. J Dent Educ *69*, 1148-1160.

Carver, C. S., Pozo, C., Harris, S. D., Noriega, V., Scheier, M. F., Robinson, D. S., Ketcham, A. S., Moffat, F. L., Jr., and Clark, K. C. (1993). How coping mediates the effect of optimism on distress: a study of women with early stage breast cancer. J Pers Soc Psychol *65*, 375-390.

Carver, C. S., Scheier, M. F., and Weintraub, J. K. (1989). Assessing coping strategies: a theoretically based approach. J Pers Soc Psychol *56*, 267-283.

Cecchini, J. J., and Friedman, N. (1987). First-year dental students: relationship between stress and performance. Int J Psychosom *34*, 17-19.

Clow, A., Thorn, L., Evans, P., and Hucklebridge, F. (2004). The awakening cortisol response: methodological issues and significance. Stress *7*, 29-37.

Cobb, S. (1976). Presidential Address-1976. Social support as a moderator of life stress. Psychosom Med *38*, 300-314.

Cohen, S., and William, G. (1988). Perceived stress in a probability sample of the United States. In The social psychology of health, S. Spacapan, and S. Oskamp, eds. (Newbury Park, CA), pp. 31.

Cooper, C., Cooper, R., and Eaker, L. (1988). Living with stress (New York, Penguin Books).

Cooper CL, W. J., Kelly M. (1987). Job satisfaction, mental health, and job stressors among general dental practitioners in the UK. Br Dent J *162*, 77-81.

Coyne, J. C., and Downey, G. (1991). Social factors and psychopathology: stress, social support, and coping processes. Annu Rev Psychol *42*, 401-425.

Czeisler, C. A., Ede, M. C., Regestein, Q. R., Kisch, E. S., Fang, V. S., and Ehrlich, E. N. (1976). Episodic 24-hour cortisol secretory patterns in patients awaiting elective cardiac surgery. J Clin Endocrinol Metab *42*, 273-283.

Dahlgren, A., Kecklund, G., and Akerstedt, T. (2005). Different levels of work-related stress and the effects on sleep, fatigue and cortisol. Scand J Work Environ Health *31*, 277-285.

De Vente, W., Olff, M., Van Amsterdam, J. G., Kamphuis, J. H., and Emmelkamp, P. M. (2003). Physiological differences between burnout patients and healthy controls: blood pressure, heart rate, and cortisol responses. Occup Environ Med *60 Suppl 1*, i54-61.

Diehl, M., Coyle, N., and Labouvie-Vief, G. (1996). Age and sex differences in strategies of coping and defense across the life span. Psychol Aging *11*, 127-139.

Dimsdale, J. E., Hackett, T. P., Block, P. C., and Hutter, A. M., Jr. (1978). Emotional correlates of type A behavior pattern. Psychosom Med *40*, 580-583.

Edwards, S., Clow, A., Evans, P., and Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. Life Sci *68*, 2093-2103.

Ellison, S. A., Mashimo, P. A., and Mandel, I. D. (1960). Immunochemical studies of human saliva. I. The demonstration of serum proteins in whole and parotid saliva. J Dent Res *39*, 892-898.

Esterling, B. A., Antoni, M. H., Kumar, M., and Schneiderman, N. (1993). Defensiveness, trait anxiety, and Epstein-Barr viral capsid antigen antibody titers in healthy college students. Health Psychol *12*, 132-139.

Evans, P., Bristow, M., Hucklebridge, F., Clow, A., and Pang, F. Y. (1994). Stress, arousal, cortisol and secretory immunoglobulin A in students undergoing assessment. Br J Clin Psychol *33 (Pt 4)*, 575-576.

Evans, P., Bristow, M., Hucklebridge, F., Clow, A., and Walters, N. (1993). The relationship between secretory immunity, mood and life-events. Br J Clin Psychol *32 (Pt 2)*, 227-236.

Faculty of Dentistry, undergraduate entry requirements. Available from: http://www.dentistry.nus.edu.sg/academic/entry.htm.

Feldman, P. J., Cohen, S., Lepore, S. J., Matthews, K. A., Kamarck, T. W., and Marsland, A. L. (1999). Negative emotions and acute physiological responses to stress. Ann Behav Med *21*, 216-222; discussion 223-216.

Fleming, R., Baum, A., Gisriel, M. M., and Gatchel, R. J. (1982). Mediating influences of social support on stress at Three Mile Island. J Human Stress *8*, 14-22.

Folkman, S., Lazarus, R. S., Dunkel-Schetter, C., DeLongis, A., and Gruen, R. J. (1986). Dynamics of a stressful encounter: cognitive appraisal, coping, and encounter outcomes. J Pers Soc Psychol *50*, 992-1003.

Fones, C. (1996). The vulnerable person. In Controlling stress, E. H. Kua, S. M. Ko, and K. Ung, eds. (Singapore, Department of Psychological Medicine, National University Hospital).

Garbee, W. H., Jr. (1981). Sources of stress in the dental school environment. Lda J *39*, 9-14.

Garbee, W. H., Jr., Zucker, S. B., and Selby, G. R. (1980). Perceived sources of stress among dental students. J Am Dent Assoc *100*, 853-857.

Garde, A. H., and Hansen, A. M. (2005). Long-term stability of salivary cortisol. Scand J Clin Lab Invest *65*, 433-436.

George, J. M., Whitworth, D. E., Sturdevant, J. R., and Lundeen, T. F. (1987). Correlates of dental student stress. J Dent Educ *51*, 481-485.

Goldberg, D., and Williams, P. (1988). User's Guide to the General Health Questionnaire (Windsor, UK, NFER-Nelson).

Goldstein, M. B. (1979). Sources of stress and interpersonal support among first-year dental students. J Dent Educ *43*, 625-629.

Goodkin, K., Blaney, N. T., Feaster, D., Fletcher, M. A., Baum, M. K., Mantero-Atienza, E., Klimas, N. G., Millon, C., Szapocznik, J., and Eisdorfer, C. (1992a). Active coping style is associated with natural killer cell cytotoxicity in asymptomatic HIV-1 seropositive homosexual men. J Psychosom Res *36*, 635-650.

Goodkin, K., Fuchs, I., Feaster, D., Leeka, J., and Rishel, D. D. (1992b). Life stressors and coping style are associated with immune measures in HIV-1 infection--a preliminary report. Int J Psychiatry Med *22*, 155-172.

Guthrie, E., Black, D., Bagalkote, H., Shaw, C., Campbell, M., and Creed, F. (1998). Psychological stress and burnout in medical students: a five-year prospective longitudinal study. J R Soc Med *91*, 237-243.

Guyton, A. (1991). Secretory functions of the alimentary tract. In Textbook of Medical Physiology (Philadelphia, Saunders), pp. 711.

Haines, V. A., Hurlbert, J. S., and Zimmer, C. (1991). Occupational stress, social support, and buffer hypothesis. Work and Occupations *18*, 212-235.

Heath, J. R., Macfarlane, T. V., and Umar, M. S. (1999). Perceived sources of stress in dental students. Dent Update *26*, 94-98, 100.

Hendricks, S. J., Joshi, A., Crombie, K., and Moola, M. H. (1994). Perceived sources of stress among black dental students in South Africa. J Dent Educ *58*, 406-410.

Henning, K., Ey, S., and Shaw, D. (1998). Perfectionism, the imposter phenomenon and psychological adjustment in medical, dental, nursing and pharmacy students. Med Educ *32*, 456-464.

Henningsen, G. M., Hurrell, J. J., Jr., Baker, F., Douglas, C., MacKenzie, B. A., Robertson, S. K., and Phipps, F. C. (1992). Measurement of salivary immunoglobulin A as an immunologic biomarker of job stress. Scand J Work Environ Health *18 Suppl 2*, 133-136.

Hinkle, L. E., Jr. (1974). The concept of "stress" in the biological and social sciences. Int J Psychiatry Med *5*, 335-357.

Holahan, C. J., and Moos, R. H. (1982). Social support and adjustment: predictive benefits of social climate indices. Am J Community Psychol *10*, 403-415.

Holahan, C. J., and Moos, R. H. (1986). Personality, coping, and family resources in stress resistance: a longitudinal analysis. J Pers Soc Psychol *51*, 389-395.

Hucklebridge, F., Lambert, S., Clow, A., Warburton, D. M., Evans, P. D., and Sherwood, N. (2000). Modulation of secretory immunoglobulin A in saliva; response to manipulation of mood. Biol Psychol *53*, 25-35.

Humphris, G., Blinkhorn, A., Freeman, R., Gorter, R., Hoad-Reddick, G., Murtomaa, H., O'Sullivan, R., and Splieth, C. (2002). Psychological stress in undergraduate dental students: baseline results from seven European dental schools. Eur J Dent Educ *6*, 22-29.

Jemmott, J. B., 3rd, Borysenko, J. Z., Borysenko, M., McClelland, D. C., Chapman, R., Meyer, D., and Benson, H. (1983). Academic stress, power motivation, and decrease in secretion rate of salivary secretory immunoglobulin A. Lancet *1*, 1400-1402.

Jolles, P., and Jolles, J. (1984). What's new in lysozyme research? Always a model system, today as yesterday. Mol Cell Biochem *63*, 165-189.

Jones, K. V., Copolov, D. L., and Outch, K. H. (1986). Type A, test performance and salivary cortisol. J Psychosom Res *30*, 699-707.

Kiecolt-Glaser, J. K., Glaser, R., Gravenstein, S., Malarkey, W. B., and Sheridan, J. (1996). Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc Natl Acad Sci U S A *93*, 3043-3047.

Kirschbaum, C., and Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology *19*, 313-333.

Kirschbaum, C., Klauer, T., Filipp, S. H., and Hellhammer, D. H. (1995). Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. Psychosom Med *57*, 23-31.

Kittel, F., Kornitzer, M., De Backer, G., Dramaix, M., Sobolski, J., Degre, S., and Denolin, H. (1983). Type A in relation to job-stress, social and bioclinical variables: the Belgian Physical Fitness Study. J Human Stress *9*, 37-45.

Knight, J. (1981). Doctor-To-Be: Coping with the Trials and Triumphs of Medical School. (New York, Appleton-Century-Crofts).

Knudsen, W. (1978). Quality of life of the dental student. Tandlakartidningen *70*, 183-186.

Ko, S. M., Kua, E. H., and Fones, C. S. (1999). Stress and the undergraduates. Singapore Med J *40*, 627-630.

Kugler, J., Hess, M., and Haake, D. (1992). Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. J Clin Immunol *12*, 45-49.

Kunz-Ebrecht, S. R., Kirschbaum, C., and Steptoe, A. (2004). Work stress, socioeconomic status and neuroendocrine activation over the working day. Soc Sci Med *58*, 1523-1530.

Lacey, K., Zaharia, M. D., Griffiths, J., Ravindran, A. V., Merali, Z., and Anisman, H. (2000). A prospective study of neuroendocrine and immune alterations associated with the stress of an oral academic examination among graduate students. Psychoneuroendocrinology *25*, 339-356.

LaRocco, J. M., House, J. S., and French, J. R., Jr. (1980). Social support, occupational stress, and health. J Health Soc Behav *21*, 202-218.

Lazarus, R. S., and Folkman, S. (1984). Stress, appraisal, and coping (New York, Springer).

LeBlanc, J., and Ducharme, M. B. (2005). Influence of personality traits on plasma levels of cortisol and cholesterol. Physiol Behav *84*, 677-680.

Lloyd, C., and Musser, L. A. (1985). Stress in dental students. J Am Coll Dent *52*, 11-19.

Malik, S. (2000). Students, tutors and relationships: the ingredients of a successful student support scheme. Med Educ *34*, 635-641.

Maruyama, S., and Morimoto, K. (1997). The effects of lifestyle and Type A behavior on the life-stress process. Environmental health and preventive medicine *2*, 28-34.

McClelland, D. C., Floor, E., Davidson, R. J., and Saron, C. (1980). Stressed power motivation, sympathetic activation, immune function, and illness. J Human Stress *6*, 11-19.

McClelland, D. C., Ross, G., and Patel, V. (1985). The effect of an academic examination on salivary norepinephrine and immunoglobulin levels. J Human Stress *11*, 52-59.

McDowell, I., and Newell, C. (1996). Measuring Health: A guide to rating scales and questionnaires., 2nd edition edn (New York, Oxford University Press).

Meeran, K., Hattersley, A., Mould, G., and Bloom, S. R. (1993). Venepuncture causes rapid rise in plasma ACTH. Br J Clin Pract *47*, 246-247.

Mestecky, J. (1993). Saliva as a manifestation of the common mucosal immune system. Ann N Y Acad Sci *694*, 184-194.

Miletic, I. D., Schiffman, S. S., Miletic, V. D., and Sattely-Miller, E. A. (1996). Salivary IgA secretion rate in young and elderly persons. Physiol Behav *60*, 243-248.

Miller, C. S., Dembo, J. B., Falace, D. A., and Kaplan, A. L. (1995). Salivary cortisol response to dental treatment of varying stress. Oral Surg Oral Med Oral Pathol Oral Radiol Endod *79*, 436-441.

Moffat, K. J., McConnachie, A., Ross, S., and Morrison, J. M. (2004). First year medical student stress and coping in a problem-based learning medical curriculum. Med Educ *38*, 482-491.

Molassiotis, A., Van Den Akker, O. B., Milligan, D. W., and Goldman, J. M. (1997). Symptom distress, coping style and biological variables as predictors of survival after bone marrow transplantation. J Psychosom Res *42*, 275-285.

Mosley, T. H., Jr., Perrin, S. G., Neral, S. M., Dubbert, P. M., Grothues, C. A., and Pinto, B. M. (1994). Stress, coping, and well-being among third-year medical students. Acad Med *69*, 765-767.

Moutsopoulos, H. M., Webber, B. L., Pavlidis, N. A., Fostiropoulos, G., Goules, D., and Shulman, L. E. (1980). Diffuse fasciitis with eosinophilia. A clinicopathologic study. Am J Med *68*, 701-709.

Mozer, J. E., Lloyd, C., and Puente, E. S. (1990). The relationship of Bi/Polar personality patterns with self-esteem, stress, and satisfaction in dental school. J Dent Educ *54*, 153-157.

Musser, L. A., and Lloyd, C. (1985). The relationship of marital status and living arrangement to stress among dental students. J Dent Educ *49*, 573-578.

Naidu, R. S., Adams, J. S., Simeon, D., and Persad, S. (2002). Sources of stress and psychological disturbance among dental students in the West Indies. J Dent Educ *66*, 1021-1030.

Nelson, N. G., Dell'Oliver, C., Koch, C., and Buckler, R. (2001). Stress, coping, and success among graduate students in clinical psychology. Psychol Rep *88*, 759-767.

Newbury-Birch, D., Lowry, R. J., and Kamali, F. (2002). The changing patterns of drinking, illicit drug use, stress, anxiety and depression in dental students in a UK dental school: a longitudinal study. Br Dent J *192*, 646-649.

Newton, J. T., Baghaienaini, F., Goodwin, S. R., Invest, J., Lubbock, M., and Marouf Saghakhaneh, N. (1994). Stress in dental school: a survey of students. Dent Update *21*, 162-164.

Ng, V., Koh, D., Chan, G., Ong, H. Y., Chia, S. E., and Ong, C. N. (1999). Are salivary immunoglobulin A and lysozyme biomarkers of stress among nurses? J Occup Environ Med *41*, 920-927.

Ng, V., Koh, D., and Chia, S. E. (2003a). Examination stress, salivary cortisol, and academic performance. Psychol Rep *93*, 1133-1134.

Ng, V., Koh, D., Fu, Q., and Chia, S. E. (2003b). Effects of storage time on stability of salivary immunoglobulin A and lysozyme. Clin Chim Acta *338*, 131-134.

Ng, V., Koh, D., Mok, B. Y., Chia, S. E., and Lim, L. P. (2003c). Salivary biomarkers associated with academic assessment stress among dental undergraduates. J Dent Educ *67*, 1091-1094.

Ohira, H., Watanabe, Y., Kobayashi, K., and Kawai, M. (1999). The type A behavior pattern and immune reactivity to brief stress: change of volume of secretory immunoglobulin A in saliva. Percept Mot Skills *89*, 423-430.

Pau, A. K., and Croucher, R. (2003). Emotional intelligence and perceived stress in dental undergraduates. J Dent Educ *67*, 1023-1028.

Pau, A. K., Croucher, R., Sohanpal, R., Muirhead, V., and Seymour, K. (2004). Emotional intelligence and stress coping in dental undergraduates--a qualitative study. Br Dent J *197*, 205-209.

Perera, S., Sabin, E., Nelson, P., and Lowe, D. (1998). Increases in salivary lysozyme and IgA concentrations and secretory rates independent of salivary flow rates following viewing of humorous videotape. Int J Behav Med *5*, 118-128.

Perera, S., Uddin, M., and Hayes, J. A. (1997). Salivary lysozyme: a noninvasive marker for the study of the effects of stress of natural immunity. Int J Behav Med *4*, 170-178.

Peretz, B., and Mann, J. (2000). Dental anxiety among Israeli dental students: a 4-year longitudinal study. Eur J Dent Educ *4*, 133-137.

Peretz, B., Rosenblum, A., and Zadik, D. (1997). Stress levels and related variables among dental students in Jerusalem, Israel. Eur J Dent Educ *1*, 162-166.

Polychronopoulou, A., and Divaris, K. (2005). Perceived sources of stress among Greek dental students. J Dent Educ *69*, 687-692.

Pruessner, J. C., D. H. Hellhammer, et al. (1999). Burnout, perceived stress, and cortisol responses to awakening. Psychosom Med **61**(2): 197-204.

Rajab, L. D. (2001). Perceived sources of stress among dental students at the University of Jordan. J Dent Educ *65*, 232-241.

Rankin, J. A., and Harris, M. B. (1990). Comparison of stress and coping in male and female dentists. J Dent Pract Adm *7*, 166-172.

Rhodes, P., and Swedlow, D. (1983). A dental student tutor program. J Dent Educ *47*, 325-328.

Rosli, T. I., Abdul Rahman, R., Abdul Rahman, S. R., and Ramli, R. (2005). A survey of perceived stress among undergraduate dental students in Universiti Kebangsaan Malaysia. Singapore Dent J *27*, 17-22.

Rospenda, K. M., Halpert, J., and Richman, J. A. (1994). Effects of social support on medical students' performances. Acad Med *69*, 496-500.

Sanders, A. E., and Lushington, K. (1999). Sources of stress for Australian dental students. J Dent Educ *63*, 688-697.

Sanders, A. E., and Lushington, K. (2002). Effect of perceived stress on student performance in dental school. J Dent Educ *66*, 75-81.

Sapolsky, R. (1999). Chapter 2: Glands, Gooseflesh, and Hormones. In Why Zebra Don't Get Ulcer. An updated guide to stress, stress-related diseases, and coping (New York, W.H. Freeman and Company), pp. 31-34.

Seeman, T. E. (1996). Social ties and health: the benefits of social integration. Ann Epidemiol *6*, 442-451.

Sewitch, M. J., Abrahamowicz, M., Bitton, A., Daly, D., Wild, G. E., Cohen, A., Katz, S., Szego, P. L., and Dobkin, P. L. (2001). Psychological distress, social support, and disease activity in patients with inflammatory bowel disease. Am J Gastroenterol *96*, 1470-1479.

Sgan-Cohen, H. D. (1989). Stress among Israeli dental students: a two-year longitudinal study. Int J Psychosom *36*, 69-71.

Smith, M. C., and Dust, M. C. (2006). An exploration of the influence of dispositional traits and appraisal on coping strategies in african american college students. J Pers *74*, 145-174.

Smyth, J. M., Ockenfels, M. C., Gorin, A. A., Catley, D., Porter, L. S., Kirschbaum, C., Hellhammer, D. H., and Stone, A. A. (1997). Individual differences in the diurnal cycle of cortisol. Psychoneuroendocrinology *22*, 89-105.

Sofola, O. O., and Jeboda, S. O. (2006). Perceived sources of stress in Nigerian dental students. Eur J Dent Educ *10*, 20-23.

Stansfeld, S. A., and Marmot, M. G. (1992). Social class and minor psychiatric disorder in British Civil Servants: a validated screening survey using the General Health Questionnaire. Psychol Med *22*, 739-749.

Stanton, A. L., and Snider, P. R. (1993). Coping with a breast cancer diagnosis: a prospective study. Health Psychol *12*, 16-23.

Steptoe, A., Cropley, M., Griffith, J., and Kirschbaum, C. (2000). Job strain and anger expression predict early morning elevations in salivary cortisol. Psychosom Med *62*, 286-292.

Steptoe, A., Kunz-Ebrecht, S., Owen, N., Feldman, P. J., Willemsen, G., Kirschbaum, C., and Marmot, M. (2003). Socioeconomic status and stress-related biological responses over the working day. Psychosom Med *65*, 461-470.

Stone, A. A., Cox, D. S., Valdimarsdottir, H., and Neale, J. M. (1987). Secretory IgA as a measure of immunocompetence. J Human Stress *13*, 136-140.

Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., and Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. Psychoneuroendocrinology *26*, 295-306.

Sturdevant, J. R., George, J. M., and Lundeen, T. F. (1987). An interactional view of dental student stress. J Dent Educ *51*, 246-249.

Sugiura, G., Shinada, K., and Kawaguchi, Y. (2005). Psychological well-being and perceptions of stress amongst Japanese dental students. Eur J Dent Educ *9*, 17-25.

Suls, J., Gastorf, J. W., and Witenberg, S. H. (1979). Life events, psychological distress and the Type A coronary-prone behavior pattern. J Psychosom Res *23*, 315-319.

Supe, A. N. (1998). A study of stress in medical students at Seth G.S. Medical College. J Postgrad Med *44*, 1-6.

Tamres, L., Janicki, D., and Helgeson, V. (2002). Sex differences in coping behavior: A meta-analytic review and an examination of relative coping. Pers Soc Psychol Rev *6*, 2-30.

Tartaro, J., Luecken, L. J., and Gunn, H. E. (2005). Exploring heart and soul: effects of religiosity/spirituality and gender on blood pressure and cortisol stress responses. J Health Psychol *10*, 753-766.

Thoits, P. A. (1995). Stress, coping, and social support processes: where are we? What next? J Health Soc Behav *Spec No*, 53-79.

Tisdelle, D. A., Hansen, D. J., St Lawrence, J. S., and Brown, J. C. (1984). Stress management training for dental students. J Dent Educ *48*, 196-202.

Tomasi, T. (1994). Introduction: an overview of the mucosal system. In Handbook of mucosal immunology, P. Orga, W. Strober, J. Mestecky, J. McGhee, M. Lamm, and J. Bienenstock, eds. (San Diego, Academic Press, Inc.,), pp. 3-8.

Tomasi, T. B., Jr., and Zigelbaum, S. (1963). The Selective Occurence of Gamma-1a Globulins in Certain Body Fluids. J Clin Invest *42*, 1552-1560.

Tsujita, S., and Morimoto, K. (1999). Secretory IgA in saliva can be a useful stress marker. Environmental health and preventive medicine *4*, 1-8.

Uchino, B. N., Cacioppo, J. T., and Kiecolt-Glaser, J. K. (1996). The relationship between social support and physiological processes: a review with emphasis on underlying mechanisms and implications for health. Psychol Bull *119*, 488-531.

Vander, A., Sherman, J., and Luciano, D. (2001). Human physiology : the mechisms of body function., 8th edn (Boston, McGraw-Hill).

Waldron, I., Hickey, A., McPherson, C., Butensky, A., Gruss, L., Overall, K., Schmader, A., and Wohlmuth, D. (1980). Type A behavior pattern: relationship to variation in blood pressure, parental characteristics, and academic and social activities of students. J Human Stress *6*, 16-27.

Wang, H. H., Wu, S. Z., and Liu, Y. Y. (2003). Association between social support and health outcomes: a meta-analysis. Kaohsiung J Med Sci *19*, 345-351.

Waugh, R. F. (2003). Measuring coping at a university using a Rasch model. J Appl Meas *4*, 370-385.

Weinberg, A., and Creed, F. (2000). Stress and psychiatric disorder in healthcare professionals and hospital staff. Lancet *355*, 533-537.

Westerman, G. H., Grandy, T. G., Lupo, J. V., and Mitchell, R. E. (1986). Relationship of stress and performance among first-year dental students. J Dent Educ *50*, 264-267.

Westerman, G. H., Grandy, T. G., Ocanto, R. A., and Erskine, C. G. (1993). Perceived sources of stress in the dental school environment. J Dent Educ *57*, 225-231.

Wexler, M. (1978). Mental health and dental education. J Dent Educ *42*, 74-77.

Yang, Y., D. Koh, Ng, V, Lee, C.Y., Chan, G., Dong, F. and Chia, S.E. (2001). Salivary cortisol levels and work-related stress among emergency department nurses. J Occup Environ Med *43*, 1011-1018.

Yang, Y., Koh, D., Ng, V., Lee, C. Y., Chan, G., Dong, F., Goh, S. H., Anantharaman, V., and Chia, S. E. (2002). Self perceived work related stress and the relation with salivary IgA and lysozyme among emergency department nurses. Occup Environ Med *59*, 836-841.

Yap, A. U., Bhole, S., and Teo, C. S. (1996). A cross-cultural comparison of perceived sources of stress in the dental school environment. J Dent Educ *60*, 459-464.

Yeh, C. K., Dodds, M. W., Zuo, P., and Johnson, D. A. (1997). A population-based study of salivary lysozyme concentrations and candidal counts. Arch Oral Biol *42*, 25-31.

Zeier, H., Brauchli, P., and Joller-Jemelka, H. I. (1996). Effects of work demands on immunoglobulin A and cortisol in air traffic controllers. Biol Psychol *42*, 413-423.

Faculty of Medicine
Faculty of Dentistry

National University of Singapore

Date:_____ | Case No | | | | |

A Prospective Study of Salivary Biomarkers and Major Life Events

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The objectives of this project are: (i) to study the stress patterns of dental students throughout the academic year
(ii) to investigate the relationship of various salivary
biomarkers with major life events

Please answer the questionnaire honestly and in sequence. The information given is for research purposes only and will be kept strictly confidential. Only members of the research team will have access to these data and any research reports will include only group level data that cannot be traced to the individual.

Please do not write your name on the questionnaire, instead please fill up the **Case No**. box with the code number allocated to you. This is to help us maintain confidentiality. If you have any queries or difficulties, please feel free to call me.

When you have completed the questionnaires, please keep it in a sealed envelope and return it to **Dr Betty Mok** or myself.

Once again, thank you.

Sincerely,
Vivian Ng
Department of Community, Occupational and Family Medicine
Faculty of Medicine (MD3)
16 Medical Drive
National University of Singapore
Singapore 117597

Tel: 874 4992 (O)   Fax: 779 1489
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I.   PERSONAL DATA

Where applicable, please fill in the blank with your answer or circle the answer of your choice.

1.   Which academic year are you in?
   a.   1st year undergraduate   b.   2nd year undergraduate
   c.   3rd year undergraduate   d.   4th year undergraduate
   e.   Postgraduate study, which year?_____

2.   Gender
   a.   Male   b. Female

3.   Date of Birth:_____

4.   Ethic group
   a.   Chinese   b.   Malay
   c.   Indian   d.   Others. Please specify:_____

5.   Are you Singaporean?   a. Yes   b.No

If 'No',   (i) Are you a permanent resident?   a.Yes   b. No

           (ii)Which country are you from?_____

           (iii) How long have you been in Singapore_____years

6.   Marital status
   a.   Single   b.   Married
   c.   Divorced / Separated   d.   Widowed

7.   Religion
   a.   Buddhism   b.   Christianity
   c.   Hinduism   d.   Islam
   e.   No religion   f.   Other
      religion:_____

8.   Place of residence
   a.   Parent's house/ Own house   b. Hostel
   c.   Rented room   d. Rented apartment
   d.   Relative's place   e. Others:_____

II. GENERAL INFORMATION

1. Do you smoke?
   - a. No
   - b. Yes
   - c. Ex-smoker (stopped >1 yr.)

   If Yes, number of sticks per day:_____
   For how long?_____years

2. How many drinks* do you usually take per week?
   - a. No
   - b. Yes, _____drinks

   *(1 drink = 1 can of beer, 1 peg/small cup of whisky, gin and other alcoholic drinks)

3. Do you exercise? a. No   b. Yes
   If yes, what type of exercise do you do? (you can choose more than one option)
   - a. aerobics
   - b. cycling
   - c. running
   - d. swimming
   - e. walking
   - e. others_____

4. On average, how many hours of continuous sleep do you get in a day?
   _____hours

5. Are you currently taking any medicine? (either prescriptive or over-the-counter medicine)
   - a. No
   - b. Yes (what medicine? please specify_____)

6. Please consider your life at the present moment and indicate how you feel about each one in turn on a scale of 0 (extremely dissatisfied) to 4 (extremely satisfied)

	0<------------------------------>4 Extremely<------------>Extremely dissatisfied satisfied				
a.The house or flat you live	0	1	2	3	4
b. Your standard of living: the things you can buy and do	0	1	2	3	4
c. Your present states of health	0	1	2	3	4
d.The education/training you have received	0	1	2	3	4
e. What you are accomplishing in life	0	1	2	3	4
f.What the future seems to hold for you	0	1	2	3	4
g. Your social status / prestige	0	1	2	3	4
h. Your social life	0	1	2	3	4
i. Your family life	0	1	2	3	4
j. How satisfied are you with your life as a whole	0	1	2	3	4

III. PERSONALITY

Circle one number for each of the fourteen questions below, which best reflects the way you behave in your everyday life. For example, if you are always on time for appointments, on question 1 you would circle a number between 7 and 11. If you are usually more casual about appointments you would circle one of the lower numbers between 1 and 5.

1	Casual about appointments	1 2 3 4 5 6 7 8 9 10 11	Never late
2	Not competitive	1 2 3 4 5 6 7 8 9 10 11	Very competitive
3	Good listener	1 2 3 4 5 6 7 8 9 10 11	Anticipates what others are going to say (nods, attempts to finish for them)
4	Never feels rushed (even under pressure)	1 2 3 4 5 6 7 8 9 10 11	Always rushed
5	Can wait patiently	1 2 3 4 5 6 7 8 9 10 11	Impatient while waiting
6	Takes things one at a time	1 2 3 4 5 6 7 8 9 10 11	Tries to do many things at once, thinks about what will do next
7	Slow deliberate talker	1 2 3 4 5 6 7 8 9 10 11	Emphatic in speech, fast and forceful
8	Cares about satisfying him/herself no matter what others may think	1 2 3 4 5 6 7 8 9 10 11	Wants good job recognized by others
9	Slow doing things	1 2 3 4 5 6 7 8 9 10 11	Fast (eating, walking)
10	Easy going	1 2 3 4 5 6 7 8 9 10 11	Hard driving (pushing yourself and others)
11	Expresses feelings	1 2 3 4 5 6 7 8 9 10 11	Hides feelings
12	Many outside interests	1 2 3 4 5 6 7 8 9 10 11	Few interests outside work/home
13	Unambitious	1 2 3 4 5 6 7 8 9 10 11	Ambitious
14	Casual	1 2 3 4 5 6 7 8 9 10 11	Eager to get things done

IV. COPING STRATEGIES

There are many ways to try to deal with difficult events in life, below is a list of how people respond when they confront difficult or stressful events in their lives. This section requires you to indicate what you generally do and feel when you experience stressful events. Of course, different events require a different set of responses, but think about what you usually do when you are faced with a difficult situation or when you are under stress.
*Please **Circle** the most appropriate choice to indicate how you often do or feel as the items presented.*

		1 Never	2 Almost never	3 Sometime	4 Fairly often	5 Very often
1.	I take additional action to try to get rid of the problem	1	2	3	4	5
2.	I try to come up with a strategy about what to do	1	2	3	4	5
3.	I put aside other activities in order to concentrate on this	1	2	3	4	5
4.	I force myself to wait for the right time to do something	1	2	3	4	5
5.	I ask people who have had similar experiences what they did	1	2	3	4	5
6.	I talk to someone about how I feel	1	2	3	4	5
7.	I look for something good in what is happening	1	2	3	4	5
8.	I learn to accept it	1	2	3	4	5
9.	I seek God's help	1	2	3	4	5
10.	I get upset and let my emotions out	1	2	3	4	5
11.	I refuse to believe that it has happened	1	2	3	4	5
12.	I give up the attempt to get what I want	1	2	3	4	5
13.	I turn to work on other substitute activities to take my mind off things	1	2	3	4	5
14.	I concentrate all my efforts on doing something about it	1	2	3	4	5
15.	I make a plan of action	1	2	3	4	5
16.	I focus on dealing with this problem, if necessary let other things slide a little	1	2	3	4	5

	1 Never	2 Almost never	3 Sometime	4 Fairly often	5 Very often
17. I hold off doing anything about it until the situation permits	**1**	**2**	**3**	**4**	**5**
18. I try to get advice from someone about what to do	**1**	**2**	**3**	**4**	**5**
19. I try to get emotional support from friends or relatives	**1**	**2**	**3**	**4**	**5**
20. I try to see it in a different light, to make it seem more positive	**1**	**2**	**3**	**4**	**5**
21. I accept that this has happened and that it can't be changed	**1**	**2**	**3**	**4**	**5**
22. I put my trust in God	**1**	**2**	**3**	**4**	**5**
23. I let my feelings out	**1**	**2**	**3**	**4**	**5**
24. I pretend that it hasn't really happened	**1**	**2**	**3**	**4**	**5**
25. I just give up in trying to reach my goal	**1**	**2**	**3**	**4**	**5**
26. I sleep more than usual	**1**	**2**	**3**	**4**	**5**
27. I do what has to be done, one step at a time	**1**	**2**	**3**	**4**	**5**
28. I think hard about what steps to take	**1**	**2**	**3**	**4**	**5**
29. I keep myself from being distracted by other thoughts or activities	**1**	**2**	**3**	**4**	**5**
30. I make sure not to make matters worse by acting too soon	**1**	**2**	**3**	**4**	**5**
31. I talk to someone who could do something concrete about the problem	**1**	**2**	**3**	**4**	**5**
32. I discuss my feelings with someone	**1**	**2**	**3**	**4**	**5**
33. I learn something from the experience	**1**	**2**	**3**	**4**	**5**
34. I get used to the idea that it happened	**1**	**2**	**3**	**4**	**5**
35. I try to find comfort in my religion	**1**	**2**	**3**	**4**	**5**
36. I feel a lot of emotional distress and I find myself expressing those feelings a lot	**1**	**2**	**3**	**4**	**5**

	1 Never	2 Almost never	3 Sometime	4 Fairly often	5 Very often
37. I act as though it hasn't even happened	1	2	3	4	5
38. I admit to myself that I can't deal with it, and quit trying	1	2	3	4	5
39. I go to the movies or watch TV, to think about it less	1	2	3	4	5
40. I take direct action to get around the problem	1	2	3	4	5
41. I think about how I might best handle the problem	1	2	3	4	5
42. I try hard to prevent other things from interfering with my efforts at dealing with this	1	2	3	4	5
43. I restrain myself from doing anything too quickly	1	2	3	4	5
44. I talk to someone to find out more about the situation	1	2	3	4	5
45. I get sympathy and understanding from someone	1	2	3	4	5
46. I try to grow as a person as a result of the experience	1	2	3	4	5
47. I accept the reality of the fact that it happened	1	2	3	4	5
48. I pray more than usual	1	2	3	4	5
49. I get upset, and am really aware of it	1	2	3	4	5
50. I say to myself "This isn't real"	1	2	3	4	5
51. I reduce the amount of effort I'm putting into solving the problem	1	2	3	4	5
52. I daydream of things other than the actual problem	1	2	3	4	5

*** the response scales of this questionnaire were recoded into 0, 1, 2, 3, 4 for never →
very often, respectively, for data analysis**

V. SOCIAL SUPPORT

The following questions ask about people in your environment who provide you with help or support. Please **circle** *the appropriate number.*

	1 Do not have such a person	**2** Not at all	**3** A little	**4** Some time	**5** Very much
1. **How easy is it to talk with each of the following people?**					
A. Your supervisors	1	2	3	4	5
B. Your mentor	1	2	3	4	5
C. Course-mates / colleagues at work	1	2	3	4	5
D. Your family members, friends, or relatives	1	2	3	4	5
E. Church groups or religious groups	1	2	3	4	5
F. Others:_____	1	2	3	4	5
2. **How much can each of the following people be relied on when things gets tough at work?**					
A. You supervisors	1	2	3	4	5
B. Your mentor	1	2	3	4	5
C. Course-mates / colleagues at work	1	2	3	4	5
D. Your family members, friends, or relatives	1	2	3	4	5
E. Church groups or religious groups	1	2	3	4	5
F. Others:_____	1	2	3	4	5
3. **How much is each of the following people willing to listen to your personal problems?**					
A. Your supervisors	1	2	3	4	5
B. Your mentor	1	2	3	4	5
C. Course-mates / colleagues at work.	1	2	3	4	5
D. Your family members, friends, or relatives	1	2	3	4	5
E. Church groups or religious groups	1	2	3	4	5
F. Others:_____	1	2	3	4	5

VI.   LIFE EVENTS

(a) For Dental Students and Postgraduates
*Please indicate how stressful the following events were to you for the **past two months** by circling on a scale below:*

	1 Not stressful	2 Slightly stressful	3 Moderately stressful	4 Very stressful	5 Not applicable
1. Amount of assigned classwork	1	2	3	4	5
2. Lack of cooperation by patients in their home care	1	2	3	4	5
3. Difficulty of classwork	1	2	3	4	5
4. Responsibilities for comprehensive patient care	1	2	3	4	5
5. Competition for grades	1	2	3	4	5
6. Patients being late or not showing up for their appointments	1	2	3	4	5
7. Examinations and grades	1	2	3	4	5
8. Difficulty in learning clinical procedures	1	2	3	4	5
9. Atmosphere created by clinical faculty	1	2	3	4	5
10. Relations with members of the opposite sex	1	2	3	4	5
11. Receiving criticism about work	1	2	3	4	5
12. Difficulty in learning precision manual skills required in preclinic and laboratory work	1	2	3	4	5
13. Lack of confidence to be a successful dental student	1	2	3	4	5
14. Lack of confidence to be a successful dentist	1	2	3	4	5
15. Lack of time for relaxation	1	2	3	4	5
16. Amount of cheating in dental school	1	2	3	4	5
17. Rules and regulations of the school	1	2	3	4	5

	1 Not stressful	2 Slightly stressful	3 Moderately stressful	4 Very stressful	5 Not applicable
18. Working on patients with dirty mouths	1	2	3	4	5
19. Lack of home atmosphere in living quarters	1	2	3	4	5
20 Completing graduation requirements	1	2	3	4	5
21 Having children at home	1	2	3	4	5
22 Marital adjustment problems	1	2	3	4	5
23. Expectations of dental school and what in reality it is like	1	2	3	4	5
24. Lack of input into the decision making process of the school	1	2	3	4	5
25. Fear of failing course or year	1	2	3	4	5
26 Insecurity concerning professional future	1	2	3	4	5
27. Financial responsibilities	1	2	3	4	5
28. Lack of time to do assigned school work	1	2	3	4	5
29. Considering entering some other fields of work	1	2	3	4	5
30. Forced postponement of marriage or engagement	1	2	3	4	5
31. Personal physical health	1	2	3	4	5
32. Attitudes of school toward women dental students	1	2	3	4	5
33. Necessity to postpone having children	1	2	3	4	5
34. Conflict with mate over career decision	1	2	3	4	5
35. Discrimination due to race, class status, or ethnic group	1	2	3	4	5
36. Having a dual role of wife/mother and dental student/professional	1	2	3	4	5
37. Inconsistency of feedback on your work between different instructors	1	2	3	4	5
38. Fear of being unable to catch up if left behind	1	2	3	4	5

(b) General Life Events

*Please place a tick (√) in the Yes column for the event that you had experienced for the **past two months**. For each experienced event, please indicate how upsetting the event ticked was to you by **circling** on a scale of 1 (least upset) to 10 (worst imaginable level of upset)*

1<------------------------------>10
(least upset)<-------------------- >(worst imaginable
level of upset)

Event

Yes

1.	Bought house/ Sold house	_____	1	2	3	4	5	6	7	8	9	10
2.	Move house	_____	1	2	3	4	5	6	7	8	9	10
3	Major house renovation	_____	1	2	3	4	5	6	7	8	9	10
4.	Separation from loved one	_____	1	2	3	4	5	6	7	8	9	10
5.	End of relationship	_____	1	2	3	4	5	6	7	8	9	10
6.	Got engaged/ Got married	_____	1	2	3	4	5	6	7	8	9	10
7.	Marital problem	_____	1	2	3	4	5	6	7	8	9	10
8.	Awaiting divorce/Divorce	_____	1	2	3	4	5	6	7	8	9	10
9.	Child started school/nursery	_____	1	2	3	4	5	6	7	8	9	10

Increased nursing responsibilities

10.	Problem with relatives	_____	1	2	3	4	5	6	7	8	9	10
11.	Problems with friends/neighbours	_____	1	2	3	4	5	6	7	8	9	10
12.	Pet related problems	_____	1	2	3	4	5	6	7	8	9	10
13.	Work-related problems	_____	1	2	3	4	5	6	7	8	9	10
14.	Change in nature of work	_____	1	2	3	4	5	6	7	8	9	10
15.	Increased or new bank loan/mortgage	_____	1	2	3	4	5	6	7	8	9	10
16.	Financial difficulty	_____	1	2	3	4	5	6	7	8	9	10
17.	Insurance problem `	_____	1	2	3	4	5	6	7	8	9	10
18.	Legal problem	_____	1	2	3	4	5	6	7	8	9	10
19.	Emotional or physical illness	_____	1	2	3	4	5	6	7	8	9	10

of close family or relative

| | | 1<------------------------------>10 |
| | | (least upset)<---------------------->(worst imaginable level of upset) |

Event

Yes

20.	Serious illness of close family or relative requiring hospitalization	_____ 1　2　3　4　5　6　7　8　9　10
21.	Surgical operation experienced by family member or relative	_____ 1　2　3　4　5　6　7　8　9　10
22.	Death of spouse	_____ 1　2　3　4　5　6　7　8　9　10
23.	Death of family member or relative	_____ 1　2　3　4　5　6　7　8　9　10
24.	Death of close friend	_____ 1　2　3　4　5　6　7　8　9　10
25.	Emotional or physical illness of yourself	_____ 1　2　3　4　5　6　7　8　9　10
26.	Serious illness requiring your own hospitalization	_____ 1　2　3　4　5　6　7　8　9　10
27.	Surgical operation on yourself	_____ 1　2　3　4　5　6　7　8　9　10
28.	Pregnancy	_____ 1　2　3　4　5　6　7　8　9　10
29.	Birth of baby	_____ 1　2　3　4　5　6　7　8　9　10
30.	Family member left home	_____ 1　2　3　4　5　6　7　8　9　10
31.	Difficult relationship with children	_____ 1　2　3　4　5　6　7　8　9　10
32.	Difficult relationship with parents	_____ 1　2　3　4　5　6　7　8　9　10
33.	Others:_____	1　2　3　4　5　6　7　8　9　10
34.	_____ :	1　2　3　4　5　6　7　8　9　10
35.	_____ :	1　2　3　4　5　6　7　8　9　10
36.	_____ :	1　2　3　4　5　6　7　8　9　10
37.	_____ :	1　2　3　4　5　6　7　8　9　10
38.	_____ :	1　2　3　4　5　6　7　8　9　10

VII.   PERCEIVED STRESS

*The questions in this scale ask you about your feelings and thoughts during the **last two months**. In each case, please indicate how often you felt or thought a certain way by **circling** the number, which you think most nearly applies to you.*

	1 **Never**	**2** **Almost** **never**	**3** **Sometimes**	**4** **Fairly** **often**	**5** **Very** **often**

In the last two months, how often have you

		1	**2**	**3**	**4**	**5**
1.	been upset because of something that happened unexpectedly?	1	2	3	4	5
2.	felt that you were unable to control the important things in your life?	1	2	3	4	5
3.	felt nervous and "stressed"?	1	2	3	4	5
4.	felt confident about your ability to handle your personal problems?	1	2	3	4	5
5.	felt that things were going your way?	1	2	3	4	5
6.	found that you could not cope with all the things that you had to do?	1	2	3	4	5
7.	been able to control irritations in your life?	1	2	3	4	5
8.	felt that you were on top of things?	1	2	3	4	5
9.	been angered because of things that were outside of your control?	1	2	3	4	5
10.	felt difficulties were piling up so high that you could not overcome them?	1	2	3	4	5

* the response scales of this questionnaire were recoded into 0, 1, 3, 4 for never →very often , respectively for data analysis

VIII. HEALTH OUTCOME MEASURE

Please read this carefully.

*We should like to know if you have had any medical complaints and how your health has been in general, **over the past two months**. Please answer **ALL** the following questions by **circling** the number, which you think most nearly applies to you.*

(a) Over the past two months, have you

		1 Not at all	2 No more than usual	3 Rather more than usual	4 Much more than usual
1.	been feeling in need of a good tonic?	1	2	3	4
2.	been feeling tired and in bad temper?	1	2	3	4
3.	felt that you are ill?	1	2	3	4
4.	been getting any pain in your head?	1	2	3	4
5.	been getting any feeling of tightness or pressure in your head?	1	2	3	4
6.	been having hot or cold spells?	1	2	3	4
7.	lost much sleep over worry?	1	2	3	4
8.	had difficulty in staying asleep once you get to sleep?	1	2	3	4
9.	felt constantly under strain?	1	2	3	4
10.	been easily upset and bad-tempered?	1	2	3	4
11.	been getting scared or panicky for no good reason?	1	2	3	4
12.	found everything out of your control?	1	2	3	4
13.	been feeling nervous and upset all the time?	1	2	3	4
14.	been thinking of yourself as a worthless person?	1	2	3	4
15.	felt that life is entirely hopeless?	1	2	3	4
16.	felt that life isn't worth living?	1	2	3	4
17.	found at times you couldn't do anything because your were just too nervous?	1	2	3	4
18.	found yourself wishing you were dead and away from it all?	1	2	3	4

(b) Over the past two months, have you

		1	**2**	**3**	**4**
1.	felt capable of making decisions about things?	More so than usual	Same as usual	Less so than usual	Much less than usual
2.	been able to enjoy your normal day-to-day activities?	More so than usual	Same as usual	Less so than usual	Much less than usual
3.	thought of the possibility that you might end your own life	Definitely not	I don't think so	Has crossed my mind	Definitely have
4.	found that the idea of taking your own life kept coming into your mind?	Definitely not	I don't think so	Has crossed my mind	Definitely have
5.	felt that you are playing a useful part in things?	More so than usual	Same as usual	Less useful than usual	Much less useful
6.	been managing to keep yourself busy and occupied?	More so than usual	Same as usual	Rather les than usual	Much less than usual
7.	been satisfied with the way you've carried out your task?	More Satisfied	About the same as usual	Less than usual	Much less satisfied
8.	been taking longer over the things you do?	Quicker than usual	Same as usual	Longer than usual	Much longer than usual
9.	felt on the whole you were doing things well?	Better than usual	About the same	Less well than usual	Much less well
10.	been feeling perfectly well and in good health?	Better than usual	Same as usual	Worse than usual	Much worse than usual

IX. MENTAL HEALTH MEASURE

*Below is a list of the ways you might have felt or behaved. Please indicate how often you felt this way during the **past two months** by **circling** the appropriate number.*

	1 **Rarely or** **none of** **time**	**2** **Some or** **little of** **the time**	**3** **Occasionally** **moderate amount** **of the time**	**4** **Most or** **all of the** **time**
For the past two months				
1. I was bothered by things that usually don't bother me	1	2	3	4
2. I did not feel like eating; my appetite was poor	1	2	3	4
3. I felt that I could not shake off the blues even with help from my family or friends	1	2	3	4
4. I felt that I was just as good as other people	1	2	3	4
5. I had trouble keeping my mind on what I was doing	1	2	3	4
6. I felt depressed	1	2	3	4
7. I felt that everything I did was an effort	1	2	3	4
8. I felt hopeful about the future	1	2	3	4
9. I though my life had been a failure	1	2	3	4
10. I felt fearful	1	2	3	4
11. My sleep was restless	1	2	3	4
12. I was happy	1	2	3	4
13. I talked less than usual	1	2	3	4
14. I felt lonely	1	2	3	4
15. People were unfriendly	1	2	3	4
16. I enjoyed life	1	2	3	4
17. I had crying spells	1	2	3	4
18. I felt sad	1	2	3	4
19. I felt that people dislike me	1	2	3	4
20. I could not get "going"	1	2	3	4

Thank you for your participation!

Appendix B

Subscales of Dental Environmental Stress (DES) questionnaire

	Academic Work		Clinical Factors
(1)	amount of assigned classwork	(2)	lack of cooperation by patient in their home care
(3)	difficulty of classwork	(4)	responsibilities for comprehensive patient care
(5)	competition for grade	(6)	patient being late or not showing for their appointment
(7)	examination and grade	(8)	difficulty in learning clinical procedures
(12)	concern about manual dexterity	(18)	working on patients with dirty mouths
(13)	lack of confident to be a successful dental student		
(14)	lack of confident to be a successful dentist		
(20)	complete graduation requirements		
(25)	fear of failing course or year		
(26)	insecurity concerning professional future		
(38)	fear of being unable to catch up if left behind		
	Faculty and Administrative Factors		**Personal Factors**
(9)	atmosphere created by clinical faculty	(10)	inconsistency of feedback on work between different instructors
(11)	receiving criticism about work	(15)	lack of time for relaxation
(16)	amount of cheating in dental school	(21)	having children at home
(17)	rules and regulation in dental school	(22)	marital adjustment problems
(23)	expectation of dental school and what is in reality it is like	(27)	financial responsibilities
(24)	lack of input into the decision making process of dental school	(28)	lack of time to do assigned school work
(32)	attitude of school towards women dental students	(30)	forced postponement of marriage or engagement
(35)	discrimination due to race, class status or ethnic group	(31)	personal physical health
(37)	inconsistency of feedback on work between different instructors	(33)	necessity to postpone having children
		(19)	lack of home atmosphere in living quarters
		(34)	conflict with mate over career decision
		(36)	having a dual role of spouse/parent and dental student
		(29)	considering entering some other fields of work

The responses are based on a 4-point Likert scale, with 1=not stressful, 2=slightly stressful, 3=moderately stressful, 4=very stressful, 5="not applicable"

Source: Adapted from Heath JR, MacFarlane TV, Umar MS. Perceived source of stress in dental students. Dent Update 1999; 26 : 94-100 with slight modifications.

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Case No				

A Prospective Study of Salivary Biomarkers and Major Life Events

Please answer these questions.

1. Have you had an upper respiratory tract infection (URTI) i.e. cough, cold, sore throat, or influenza in the last ONE (1) week?

    a. Currently having URTI

    b. Just recovered from URTI in last week

    c. No URTI at present or in the last one week

2. How many times have you suffered from URTI in the last two months?_____

3. How many days of sick leave have you taken in the last two months on account of URTI?_____

Instructions for Collection of Salivary Sample

Please do not eat or drink except water one hour prior to saliva collection

1. First rinse your mouth thoroughly with water and spit out completely.

2. Start timing yourself for exactly **FIVE (5) minutes**.

    During this time, **do not swallow** any saliva.

3. At the end of 5 minutes, unscrew the sterile container, and bring the container to your mouth, until it is against your lips.

4. **Gently** spit **ALL** the saliva, which has collected in the last 5 minutes into the appropriate labeled container.

Thank you!

Date:_____

Time of saliva collection:_____

Appendix D

List of papers published and conference presentations arising from this study

Papers published in international peer-reviewed journals:

1. **Ng V**, Koh D, Mok B, Lim LP Chia SE. Stress, coping strategies and salivary immunoglobulin A of dental undergraduates in Singapore. Journal of UOEH 2006, 28: 160-165

2. **Ng V**, Koh D, Mok D, Lim LP, Yang Y, Chia SE. Stressful Life Events of Dental Students and Salivary Immunoglobulin A. Int J Immunopathol and Pharmacol 2004,17,2 (S):49-56 .

3. **Ng V**, Koh D, Mok BYY, Chia SE, Lim LP. Salivary biomarkers associated with academic assessment stress among dental undergraduates. J Dent Educ 2003; 67(10): 1091-1094.

Conferences Presentations

1. **Ng V**, Koh D, Mok B, Lim LP. Stress Patterns and Its Association with Self Reported Health among Dental Students over An Academic Year has been accepted for presentation at 28th International Congress on Occupational Health, - Renewing a century of commitment to a health, safe and productive working life. Milan, Italy, 11-16 June 2006.

2. **Ng V**, Koh D, Mok B, Lim LP, SE Chia. Is There a Gender Difference in Stress Perception and Coping Strategies among Dental Undergraduates? Combined Scientific Meeting – Shaping A New Era in Healthcare-/ Raffles City Convention Centre, Singapore, 4-6 November 2005.

3. **Ng V**, Koh D, Mok B, SE Chia, Lim LP. Stress and Coping Strategies in Clinical and Non-clinical Dental Students. Second ICOH International Conference on Psychosocial Factors at Work – East Meets West - Job Stress Prevention in Global Perspective. Okayama, Japan, 23-26 August 2005

4. **Ng V**, Koh D, Mok B, Lim LP. How Dental Undergraduates Cope with Stress? Regional Conference on Occupational Health. Kuala Lumpur, Malaysia, 15-17 April 2005.

5. **Ng V**, Koh D, Mok B, Lim LP Chia SE. Stress, coping strategies and salivary immunoglobulin A of dental undergraduates in Singapore. The 6th ICOH International Conference on Occupational Health for Health Care Workers. Kitakyushu, Japan, 8-10 October 2004.

6. **Ng V**, Koh D, Mok B, Lim LP Chia SE. Perceived source of stress and coping styles among first year dental undergraduates in Singapore. 25th Stress and Anxiety Research Society Conference, Amsterdam, The Netherlands, 8-10 July 2004.

7. **Ng V**, Koh D, Mok B, Fu Q, Lim LP, Chia SE. Stress and stressors among dental undergraduates over the course of the academic year. 1st International Conference on Occupational and Environmental Health, Hanoi, Vietnam, 12-14 November 2003.

8. **Ng V**, Koh D, Fu Q, Chia SE. Effects of storage time on stability of salivary immunoglobulin A and lysozyme. 7th NUS-NUH Annual Scientific Meeting 2-3 Oct, 2003.

9. **Ng V**, Koh D, Mok B, Choo S, Lim LP. Salivary biomarkers associated with academic examination. The 17th Asian Conference on Occupational Health. Taipei, Taiwan, 1-4 November 2002.

10. **Ng V**, Koh D, Mok B, Lim LP, Yang Y, Chia SE. Stressful life events of dental students and a salivary biomarker. Congress INEWE (Immune and Neurological effects of Work and Environment) Taiyuan, China, April 21-24 2002.