



## Assessment of Salivary Biomarkers Associated with Occupational Stress

Amir Heberd<sup>1</sup>, Ahmad Razali, Sundara Rajan Mahalingam<sup>2</sup>, Chua Say Tiong<sup>3</sup>, and Kamaruddin Arshad<sup>4</sup>

Faculty of Health Science, UiTM Pulau Pinang and Faculty of Health Science, UiTM Puncak Alam

Corresponding email [amirhe2831@ppinang.uitm.edu.my](mailto:amirhe2831@ppinang.uitm.edu.my), [ahmadr2772@puncakalam.uitm.edu.my](mailto:ahmadr2772@puncakalam.uitm.edu.my), [sundararajan@ppinang.uitm.edu.my](mailto:sundararajan@ppinang.uitm.edu.my), [cstiong2830@ppinang.uitm.edu.my](mailto:cstiong2830@ppinang.uitm.edu.my), [kamaruddin.arshad@ppinang.uitm.edu.my](mailto:kamaruddin.arshad@ppinang.uitm.edu.my)

### Article Information

#### Keywords

Biomarkers, Immunoglobulin A, Alpha Amylase, Passive Drooling Technique, Perceived Stress Scale

### Abstract

This study aims to identify salivary biomarkers immunoglobulin A (SIgA) and alpha amylase (SAA) level associated with occupational stress. Additionally, this study also assessed the self-perceived stress between two working group (area) namely administration vs. production and smokers vs. non-smokers. All saliva samples for assessment of SIgA and SAA was collected between during the day using passive drooling technique and preserved at  $-20^{\circ}\text{C}$  prior to analysis. The p-value for the test of SAA between administrative and production showed no difference ( $p=0.601$ ). Meanwhile, the p-value for SAA vs. smoking status is less than 0.05. Thus, there is difference in mean for SAA ( $p=0.004$ ) between smoking and non-smoking. Higher mean of SAA level was observed in non-smoking workers compared to smoking workers. On the other hand, the p-value for test of SIgA between administration and production showed no differences ( $p=0.788$ ). In contrast, there is significant differences ( $p=0.000$ ) between SIgA level for smoking and non-smoking workers. Higher mean of SIgA level was observed in non-smoking workers compared with smoking workers. There was no correlation ( $r= -0.083$ ) between Perceived Stress Scale (PSS) and SAA level. A positive fair correlation ( $r = 0.367$ ) was obtained between PSS and SIgA level

### INTRODUCTION

Definition by (Palmer, 1989) specifically stated that stress is the psychological, physiological and behavioural response by an individual when there is unbalance between the needs and their ability to meet their needs that over some period of time could affect one's health. Therefore in simple words, stress occurs when pressure exceeds the ability to manage. Occupational stress, particularly the relationships between work-related psychosocial stressors and health is a growing concern. In response to this fact, many methods have been brought forward in assessing stress in order to decrease stress and promote wellbeing. Stress may result in immune suppression that may lead to reduced disease resistance. In animal models, reduction in disease resistance has resulted in infections, cancer, or autoimmunity, while clinical case studies have shown similar predilection for disease in humans having immunodeficiency due to excessive stress (Henningesen et al., 1992).

Over the past decades, salivary biomarker has gained a lot of interest in the detection and assessment of stress (Nater et al., 2005). This is based on the fact that saliva sample have many advantages such as wide range of constituents, non-invasive, easy and do not need trained staff (Koh & Koh, 2007). It also does not cause additional stress in sample collection unlike blood or urine collection (Takai et al., 2004). The results obtain through this study can be used in determining whether the biomarkers are reliable in assessing job related stress and the influence of smoking on biomarkers concentration. With this, the research on stress can be further explored and the prevention and treatment to stress can be implemented in order to produce a stress-free society.

## METHODOLOGY

In a cross-sectional community-based study, 60 people aged 21-50 working in a steel manufacturing plant were investigated to determine the concentration of Salivary Immunoglobulin A (SIgA) and Salivary Alpha Amylase (SAA). A total of 60 saliva samples (n=60) was obtained from workers of plant. The samples were collected in the morning, between 8.30 am and 12.00 pm without being stimulated by any sorts of material. Passive drooling method is perform by tilting the head forward, collecting saliva at the front of the mouth and then spat in to a sterile container (Strazdins, et al., 2005). Approximately, 2-3 ml of saliva is collect by passive drool into plastic vials (Vivian Ng, et al., 2003). The samples were preserved, transported to Environmental Health and Safety Department laboratory of UiTM Puncak Alam where it was at  $-20^{\circ}\text{C}$  in the freezer until further analysis.

The samples were thawed completely, vortex and centrifuge at 3000 rpm for 15 minutes prior to testing. Samples should be at room temperature before assaying. The optical density of sIgA (U/mL) and SAA (U/mL) were determined using enzyme-linked immunosorbent assay kits manufactured by Salimetrics® LLC, USA (Salimetrics & Europe, 2011). Data was analysed using ELISA Plate Reader at 420 nm wavelength. Analysis of result was performed using GraphPad Prism Version 5.04 software for Windows (GraphPad Software Inc., San Diego, CA, U.S.A). All instruments that use in this study must be calibrate and must follow the instruction from the manual like ELISA Plate Reader.

A total of 60 questionnaires (n=60) was distributed to respondents from the plant. Questionnaires which consist of perceived stress scale (PSS) and stressful life events (SLE) were used as tool to gain additional relevant information related to the study. Perceived stress was evaluated using self-administered Goldberg General Health Questionnaire (GHQ-12) (Golderberg, 1970). The GHQ is a measure of current mental health and since its development by Goldberg in the 70s; it has been extensively used in different settings and different cultures (Jacob, et al., 1997). The scale asks whether the respondent has experienced a particular symptom or behaviour recently. Each item is rated on a four-point scale (less than usual, no more than usual, rather more than usual, or much more than usual). The scoring methods are Likert scoring styles (0-1-2-3). For SLE, stressors were measured using a self-administered stressful life events questionnaire. The questionnaire has 21 items covering 4 dimensions which are financial, job security, social relation and family conflict issues. Stressful life events can affect the human body responds through activating the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis, which may in turn affect the cardiovascular, the metabolic, and the immune systems (McEwen, B. S., 1998).

All acquired data was then analyzed using descriptive and inferential statistic with Statistical Package for Science Social (SPSS) program version 17 and Microsoft Office Excel 2007. The results were analyzed by using Independent t-test and nonparametric Mann-Whitney Test.

## RESULTS AND DISCUSSION

Based on the distributed questionnaire, 50% of the respondents were from administration department and the rest were from production. The percentage remained the same for smoking and non-smoking respondents. As for the respondents age group, those between 21-30 years old made up 23%, followed by 31-40 years old 21% and 41-50 years old 16%.

Results from the questionnaire showed that 47 out of the 60 participants experienced stress while the remaining 13 respondents did not. Both administration and production workers indicated high stress level with average score 19.7 and 19.0 respectively. For stress level between smoking and non-smoking, the average score was 18.9 and 20.8 which implies that non-smokers were more stressed than smokers. In a study conducted by Feizi, et al., (2012), entitled Association of Perceived Stress with Stressful Life Events, Lifestyle and Sociodemographic Factors: A Large-Scale Community-Based Study Using Logistic Quantile Regression, it was found that although being a smoker had positive impact on more perception of stress, it was not statistically significant. However majority of previous studies that studied the relation between stress and smoking have

predominantly shown that smokers report higher stress levels than non-smokers; according to the observation of higher stress in smokers versus non-smokers, it was concluded that smoking causes stress (Parrot A.C, 2012).

Based on the study, the major stressful life event experienced by workers in this plant are job security issue and financial issue. Low salary (65%) and high responsibility job (60%) are major constituents of stress under job security issues. On the other hand, debt and lower income were found to be the potential financial factor that cause stress among the workers with 58.33% and 55.0% respectively. This finding is in concurrent with the results from Feizi, et al.'s study conducted in 2012. Lorant et al. (2003), in his research stated that the individual with low income (low economic level) experienced stress twice as compared to the one in the high economic level.

Table 1 explains the concentrations salivary alpha amylase (SAA) among the plant workers.

TABLE I  
DESCRIPTIVE ANALYSIS OF PERSONALITY TRAITS

Salivary Biomarkers	Category	Mean $\pm$ SD	Minimum Value	Maximum Value	p-value
Salivary Alpha Amylase (SAA)	Administration	138.624 $\pm$ 72.541	24.600	321.440	0.601
	Production	129.294 $\pm$ 64.468	47.500	285.360	
	Smokers	107.650 $\pm$ 55.525	24.600	271.584	*0.004
	Non-Smokers	157.429 $\pm$ 70.549	33.784	321.440	

\*p-value < 0.05, there is significant difference

From Table 1, p-value is 0.601, which is more than 0.05. Earlier studies show that salivary alpha amylase is responsive to various types of challenging situations including heat stress, socially and cognitively oriented laboratory tasks and physical exercise (Chatterton, et al., 1996; Nater, et al., 2005). Interestingly, these findings were extended by a study that revealed that alpha amylase changes in response to but not in anticipation of an athletic competition (Granger, et al., 2007).

On the other hand, mean concentrations of SAA in cigarette smokers were lower compared to the non-smoker. There are significant differences between smokers and non-smokers with p-value is 0.00, which is less than 0.01. A study of cigarette smoking on the activity of SAA by (Sariri, Ghafoori, Varasteh & Erfani, 2009) supported this present finding. Increased in SAA concentration between smokers and non-smokers was also detected by Onyesom et al., 2012 when they investigate activities of alpha amylase in serum and saliva of some Nigerian cigarette smokers.

Table II explains the concentrations of Salivary Immunoglobulin A (SIgA) among the plant workers.

TABLE II  
SALIVARY IMMUNOBLOBULIN A(SIGA) CONCERNTRATIONS AMONG STEEL MANUFACTURING WORKERS

Salivary Biomarkers	Category	Mean $\pm$ SD	Minimum Value	Maximum Value	p-value
Salivary Immunoglobulin A (SIgA)	Administration	145.577 $\pm$ 70.354	60.7	294.450	0.788
	Production	140.550 $\pm$ 73.410	47.5	293.400	
	Smokers	111.488 $\pm$ 49.427	47.5	231.200	*0.000
	Non-Smokers	174.638 $\pm$ 76.459	62.1	294.450	

\*p-value < 0.05, there is significant difference

Based on independent sample t-test, there is no significant difference for concentration of SIgA between work area  $p=0.788$ . Contrary, the p value for SIgA between smoking and non- smoking show a significant difference with p value is less than 0.01.

Chronic stress has been reported to down-regulate sIgA, whereas an acute psychological induces mobilization (Tsujita & Morimoto, 1999). In this study, the mean concentration of SIgA was slightly lower in production workers than administration workers. Thus, production workers experienced higher stress than administrative. This finding was contradicted with a study by (Stojanovic, Milenovic & Marcovic, 2012) where the administrative workers are more prone to stressful influences than their production colleagues. Factors such as communication with larger number of people, bad work conditions (noise, lightning, temperature), work in shifts, overtime work, introducing new technologies as well as ergonomic and anthropometric factors were contribute to stress development among administration workers (Stojanovic, et al., 2012).

The mean SIgA among smoking is lower compared to the non- smoking workers. In agreement, earlier study by (Bennet & Reade, 1982) show significantly decreasing of SIgA concentration when they studied SIgA levels in normal subjects, tobacco smokers and patient with minor aphthous ulceration. Decreased SIgA levels were also found by Barton et al. when they investigated mucosal immunodeficiency in smokers (Griesel & Germishuys, 1999).

Table III shows association between salivary biomarker and self perceive stress.

TABLE III  
CORRELATION BETWEEN SALIVARY BIOMARKER AND SELF PERCEIVE STRESS

Variables	P Value	R Value	Direction Of Correlation	Interpretation
PSS VS SAA	0.529	0.083	Negative	No correlation
PSS VS SIGA	0.004	0.367	Negative	Fair correlation

Fair correlation was observed between perceived stress scale and concentration of Salivary Immunoglobulin A (SIgA). The negative/ inversely proportional relationship was obtained between these two variables. In a simple word, the higher perceive stress score achieve by workers, the lower concentration of SIgA in their saliva. A probable explanation is that chronic stress suppresses the immune system. According to (Tsujita & Morimoto, 1999), chronic stress will suppress the immune system. Thus, it reduces the secretion of IgA several days after the stressor. Acute psychological challenges however increase the IgA secretion immediately after a short-term stressor. These workers are likely to expose to the chronic stress for some period of time before the study was conducted.

## RECOMMENDATION

Different people have different level of stress, but the effect is still the same. Headaches, fatigue, insomnia, stomach disorder and hypertension are some of the effects. It can be worst like depression, anxiety, compulsive behaviors, and abuse. Job stress can be reduce by prioritizing and organizing task, regular exercise of brisk walking, smile always, get enough sleep and taking a vacation. Smoke cessation program is useful tool to break the nicotine dependence habits. Moreover, it will help increasing the health of respiratory system, reduce risk or heart disease and increase fertility. Stress management programs offer help to identify the sources of stress, and learning the best way to deal with it. The counselling method were much helpful to deal with stress person by sharing and exchanging opinion and problems.

## CONCLUSION

The biomarker that shows significant result in this study could be use as potential indicator for assessing stress. There is fair correlation between Perceive Stress Scale and level of SIgA. These findings may suggest that salivary immunoglobulin A could be considered as an indicator for assessing stress of anticipation stressor. With regard to this study, these salivary biomarkers can be a possible assessment tool in recognizing stress and poor performance of workers due to inability to handle with stress. Additional studies can be done on the correlation of self-perceived stress with the levels of biomarkers with more in-depth questions and investigating and provide the baseline or level of each biomarker that could indicate one as being stressful.

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