



## Assessment of Salivary Biomarkers on Work- Related Stress

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**Abstract:** Work-related stress may adversely affect physical and psychological health. The aim of this study was to measure biomarkers of stress; cortisol, immunoglobulin A (IgA) and C-reactive protein (CRP) in saliva, among some Egyptian workers and to investigate stress associated work-related and socio-demographic factors. The study included 66 Egyptian workers (50 females and 16 males) with mean age of 38 years. Biomarkers were determined using ELISA technique and a self-reported questionnaire was designed to collect data about variables under study. Results showed significant negative correlation between age and CRP levels. CRP also showed to be significantly higher among the non-married subjects. Salivary biomarkers of stress succeeded to show significant differences among study variables. Researchers showed significantly lower cortisol level than employees group that may suggest acute state of stress.

**Keywords:** Occupational stress; Salivary Cortisol; IgA; CRP; Stress biomarkers

### 1. INTRODUCTION

Occupational stress can be defined as a negative physiological and psychological response to work-related conditions [1]. It can adversely affect physical health [2]. Individuals suffering from chronic stress could be subjected to emotional vulnerability and tendencies to experience psychosomatic symptoms [3]. According to Taravati and Kaklar [4], environmental stressors- including job stress- could even aggravate infectious diseases. Moreover, job satisfaction is greatly affected by prevalence of work stress[5] with an obvious impact on the rate of turn-over [6].

Biomarkers as diagnostic tools are believed to be objective and indicative for actual clinical outcomes of stress [7]. On biological bases experience of stressful stimuli precipitates a complex and counterbalancing set of hormonal responses in the sympathetic nervous system (SNS) and hypothalamic-pituitary-adrenal (HPA) axis [8]. Chronic stimulation of these responses causes imbalances in their functioning that is mainly characterized by cortisol secretion.

Cortisol is known to have numerous physiological effects, including glucose metabolism and immune response. Chronically elevated levels of cortisol may result in hypertension, abdominal obesity, and memory impairment, while low levels of cortisol may be associated with excessive immune responses [9]. Saliva and the biomolecules found in saliva often play important immune defense roles and can be used for non invasive screening of many systemic diseases. Salivary cortisol is a traditional marker of stress that monitors the HPA axis function [10]. Similarly, Immunoglobulin A (IgA) is another biomarker that changes in response to psychosocial stress [11]. Reductions in IgA are often indicative of a reduction in functioning of the immune system [12].

As for C-reactive protein (CRP) which is one of the best characterized systemic inflammatory biomarkers, an increase in the level of perceived stress is reported to be independently associated with a higher level of CRP [13]. A prominent increase in CRP serum level after a stressful task which showed to be more evident in persons reporting higher effort-reward imbalance at the work place [14]. Stress related to social interactions is also correlated with the level of CRP even among healthy adolescents [15]. As for the relationship between salivary and serum CRP considerable amount of recent studies reported a moderate to strong association between CRP measured in saliva and that measured in serum [16-17].

The main objective of the present study is estimation of levels of biomarkers of stress; cortisol, IgA and CRP in saliva among a pilot sample of Egyptian workers and investigation of associate work-related and socio-demographic factors.

## 2. SUBJECTS AND METHODS

Subjects included in the study are 66 (50 females and 16 males) with mean age of 38 years. Morning Saliva was collected from the study participants for biochemical assessment of markers of stress. The common technique used to collect saliva samples for assay required that participants pass saliva by spitting or drooling directly into a sterilized tube, for a few minutes until sufficient volume (approximately 2 ml) has been collected. Saliva samples were stored separately in a small polypropylene tube in freezing chamber at -20°C until analysis. Salivary cortisol level was measured using ELISA kit SLV-2930 (DRG instruments GmbH, Germany), salivary IgA level was measured by ELISA kit DRG IgA Salivary (SLV-4636, DRG instruments GmbH, Germany) and salivary CRP was measured by Human high sensitivity C- Reactive Protein (hs-CRP) ELISA kit (Glory Science Co., Ltd- 310013 P.R. China).

Participants were also asked to fill a self report questionnaire including their socio-demographic data: gender, age, monthly income (less than 1200 Egyptian pound, less than 5000 Egyptian pound or more than that) and their marital status: married, non-married or other. The questionnaire also asked about some work-related variables like their profession: employees or researchers, whether the nature of their job is mental, physical or both, how long they have been working in their jobs (less than five years, less than ten years or more than that) and the net working hours per day (less than three hours, less than five hours or more than five hours).

The protocol was approved by the Ethical Committee for medical research of the National Research Centre, Dokki, Giza, Egypt. Statistical analysis for obtained results was carried out using the statistical package for social sciences, version 20 for windows (SPSS Inc., USA). Continuous data were expressed as mean ± SD and were compared using Student’s t-test and ANOVA test. Pearson Correlation was done for testing relation between variables. Value of p<0.05 was considered statistically significant.

## 3. RESULTS

As shown in table1, females represented (76%) while males were (24%). Age ranged between 25 and 59 with 38 years as mean value. Higher percentages appeared for those working for more than 5 years (78%), researchers (69%) and the married (76%) concerning marital status.

**Table 1.** Descriptive data for study variables

Variable (N)	N (%)	
<b>Gender</b>	Male	16 (24%)
	Female	50 (76%)
<b>Social status (57)</b>	Non married	7 (12%)
	Married	43 (76%)
	Other	7 (12%)
<b>Working years (60)</b>	<5 y	13 (22%)
	<10 y	20 (33%)
	≥10 y	27 (45%)
<b>Working hours (60)</b>	< 3 hrs	3 (5%)
	<5 hrs	29 (48%)
	≥ 5 hrs	28 (47%)
<b>Monthly income (58)</b>	< 1200 LE	10 (17%)
	< 5000 LE	27 (47%)
	≥ 5000 LE	21 (36%)
<b>Job nature (61)</b>	Mental	26 (43%)
	Physical	3 (5%)
	Both	32 (52%)
<b>Profession (66)</b>	Researcher	47 (69%)
	Employee	19 (31%)

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As shown in table (2), the mean values for cortisol lies at the middle third of the reference normal range (1.2-14.7 ng/ml). As for IgA, the mean value (148.26+124.4 unit) lies at the upper limit of the reference normal range which is (40-170 µg/ml). While mean value for CRP showed to be 0.21unit.

**Table 2.** Descriptive statistics of salivary biomarkers; CRP, cortisol and IgA

	N	Minimum	Maximum	Mean	Std. Deviation
CRP	65	0.02	3.3	0.21	0.12
Salivary Cortisol (ng/ml)	63	0.20	12.4	5.02	2.19
IgA (µg /ml)	64	7.44	411.1	148	124

Biochemical parameters were compared in relation to the studied variables as shown in table 3. Researchers group showed a significant lower serum cortisol level than the group of employees (p=0.03). No statistically significant difference was shown concerning their mean levels of CRP and IgA. On the other hand, CRP showed to be extremely higher among the non-married group compared to other groups at a significant p value of less than 0.01. Male gender also showed significantly higher cortisol than females at p=0.04.

**Table 3.** Comparing means of salivary biomarkers: CRP, cortisol and IgA according to study variables

Variable	Salivary Cortisol (ng/ml)	Salivary CRP	Salivary IgA (µg/ml)
<b>Gender</b>			
Male	5.8±1.8*	0.10±0.06	145±114
Female	4.5±2.0*	0.20±0.49	157±131
<b>Social status</b>			
Non married	4.1±1.9	0.7±1.2**	114±119
Married	5.1±2.0	0.15±0.1**	157±131
Others	5.6±3.7	0.15±0.1**	132±78
<b>Working years</b>			
<5 y	5.3±1.8	0.24±0.3	118±116
<10 y	4.7±2.1	0.17±0.1	156±136
≥10 y	5.0±2.5	0.25±0.6	171±123
<b>Working hours</b>			
< 3 hrs	3.6±1.2	0.08±0.0	201±150
<5 hrs	4.6±2.2	0.26±0.6	141±118
≥ 5 hrs	5.6±2.2	0.18±0.2	154±123
<b>Monthly income</b>			
< 1200 LE	5.9±2.7	0.22±0.3	120±88
< 5000 LE	4.8±2.0	0.28±0.5	137±37
≥ 5000 LE	4.8±2.4	0.16±0.10	156±127
<b>Job nature</b>			
Mental	4.8±1.9	0.15±0.1	175±133
Physical	4.8±0.79	0.10±0.1	104±102
Both	5.1±2.6	0.29±0.6	142±121
<b>Profession</b>			
Researcher	4.6±2.3*	0.25±0.50	160±125
Employee	6.0±2.1*	0.15±0.09	127±124

\*significant at p<0.05, \*\*significant at p<0.01

As shown in table 4, significant negative correlation was detected between age and CRP.

**Table 4.** Correlation between study biomarkers as well as age

	Age (years)	Salivary CRP	Salivary Cortisol (ng/ml)	Salivary IgA (µg/ml)
<b>Age (years)</b>				
Pearson Correlation	1	<b>-0.272*</b>	0.186	0.069
Sig. (2-tailed)		<b>0.047</b>	0.187	0.625
<b>Salivary CRP</b>				
Pearson Correlation	<b>-0.272*</b>	1	-.066-	-0.064-
Sig. (2-tailed)	<b>0.047</b>		0.623	0.633
<b>Salivary Cortisol (ng/ml)</b>				
Pearson Correlation	0.186	-0.066-	1	0.064
Sig. (2-tailed)	0.187	0.623		0.637
<b>Salivary IgA (µg/ml)</b>				
Pearson Correlation	0.069	-0.064-	0.064	1
Sig. (2-tailed)	0.625	0.633	0.637	

\*Correlation is significant at the 0.05 level (2-tailed).

#### 4. DISCUSSION

Salivary biomarkers were used in the present study to investigate the effect of some socio-demographic and work-related variables on experience of stress among a pilot sample of Egyptian working adults. A growing trend is noticed that encourages saliva sampling upon measuring stress biomarkers. Saliva samples could be easily collected with the ability of self collection after giving simple instructions. In addition, saliva is more accepted than blood samples since there is no pain from needle sting with no fear of infection transfer. The non-invasive nature of saliva sampling also represents another privilege compared to blood sampling upon determination of stress biomarkers. Besides, saliva contains a wide array of components that could be used as markers and could reflect their exact level which is not the case for urine samples for example [18].

Biomarkers chosen for the present study were salivary cortisol, CRP and IgA. Salivary cortisol and CRP were chosen in particular for their known relation to stress with the advantage of having directly associated concentrations in saliva and circulating blood [19]. As for saliva IgA, it is recommended for determination of stress and could be detected in saliva independently from cortisol [20]. IgA have also become known as a highly sensitive stress biomarker that could even be used for the purposes of monitoring stress level [21].

Profession (researchers vs employees), as one of the variables under investigation, showed to have association with the study biomarkers. Researchers had significantly lower salivary cortisol level and higher values for IgA -despite being non significant- than employees that might suggest the presence of acute stress according to literature [22-26]. Yet, their morning salivary cortisol levels are still lying within the normal range (5.69 +/- 4.58 ng/ml) stated by Brandstadter et al. [27]. Putting in mind that changes in immune function in response to stress, especially IgA are sometimes bi-directional [25], there could be an alternative explanation of the significant difference between researchers and employees regarding level of salivary cortisol. The other explanation favors employees suffering from chronic stress [28]. But again, upon noticing that levels of salivary IgA for researchers is elevated (160 µg/mol) to approach the upper maximum value (170 µg/mol) among the normal range (40-170 µg/mol), it is more liable that researchers suffer from acute stress than employees being suspected for chronic distress. The elevated salivary CRP among researchers over employees also favors that researchers suffer from acute stress [29].

Social status was another variable that showed significant differences in stress biomarkers levels. The non-married group showed extremely higher mean for salivary CRP than other groups in comparison. In a similar study for assessment of stress among Egyptian working adults done by Ali et al. [30], the non-married subjects showed higher allostatic load. Social deprivation is well known for its stressful effect generally and is associated with a variety of non-desirable health responses [5,31].

In conclusion, it is highly recommended to screen stress prevalence among working adults and to figure out the different factors within the working environment that are perceived as stressful. Salivary biomarkers represented in the present study are easily measured and succeeded to show differences between groups in spite of the small sample size. Nevertheless, using a multi-component measure for detection of stress like Allostatic load index may provide better results. More investigations with larger sample could provide important output that can guide employers, decision makers and healthcare specialists.

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