Estimation of Salivary Cortisol Levels in Tobacco Cigarette Smokers

Anieta Merin Jacob[1], A.P. Indira[2], Maria Priscilla David[3], John Abraham[4], Shibu P[5]

ABSTRACT

Introduction: Cigarette smoking is associated with numerous diseases and constitutes a serious challenge to the current healthcare system worldwide. The effect of smoking on endocrine function documents that, smoking have multiple effects on hormone secretion including effects on the hypothalamic-pituitary-adrenal axis. Cortisol is the major glucocorticoid which activates the response of the body to stress conditions. The direct acute effect of smoking on cortisol levels have been attributed to activation of central nicotinic receptors. Nicotine stimulates hypothalamic cholinergic receptors leading to a release of corticotrophin releasing hormone (CRH) which in turn stimulates ACTH secretion from pituitary. ACTH hormone enters the blood stream acts on the adrenal glands, causing them to produce cortisol. Expression of cortisol in saliva may reflect HPA axis adaption to stress. With the above background this study was taken to estimate the salivary cortisol level as a stress biomarker in tobacco smokers.

Material and methods: This study was undertaken with a total sample size of 80 male patients, with an age range of 18 to 70 years. The selected subjects were divided into 2 groups: Group I (Control group) and Group II (study group). Saliva was collected from the patients (7.am to 8.a.m.) to estimate salivary cortisol. The obtained data was subjected to statistical analysis using independent Student's T -test which was used to compare the distribution of age, duration of smoking and number of cigarettes used per day in Group II . Pearson correlation co-efficient was used for comparison of salivary cortisol and stress score in Group I and Group II.

Results: The mean salivary cortisol level observed in study group (0.60) was high when compared to control group(0.28). The mean stress score observed was high in Study Group (28.7) when compared to Control Group (18.7). The correlation coefficient of salivary cortisol level and stress score observed in Study group (0.332) was high when compared to Control group (0.457). The observed difference was found to be highly statistically significant.

Conclusion: This study advocates the use of salivary cortisol level as a stress biomarker in tobacco smokers.

Keywords: Tobacco Smokers, Cortisol, Depression, Anxiety, Stress.

INTRODUCTION

Cigarette smoking is associated with numerous diseases and poses a serious challenge to the current healthcare system worldwide Tobacco smoking is most commonly seen in people with psychological problems and mental health disorders such as anxiety or alcohol abuse.[1] Smoking has an effect on the various metabolic and biological processes in the body including secretion of hormones. These are mediated chiefly through behavioral and pharmacological actions of nicotine. Recent reviews of the effect of smoking on endocrine function documents that, smoking has multiple effects on hormone secretion including effects on the hypothalamic-pituitary-adrenal axis.[2]

Cortisol is the major glucocorticoid in humans and is called as the stress hormone.[1] It activates the response of the body to stress conditions. On exposure to chronic stress the body goes through a series of events which leads to an increase in cortisol production.[3] The direct acute effect of smoking on cortisol levels have been attributed to activation of central nicotinic receptors.[2]

Nicotine stimulates hypothalamic cholinergic receptors leading to a release of corticotrophin releasing hormone (CRH) which in turn stimulates ACTH secretion from pituitary. ACTH hormone enters the blood stream, acts on the adrenal glands, causing them to produce cortisol.[3] Cortisol levels can be measured in plasma, urine and saliva.[2, 3]

Recent developments in clinical pharmacology have indicated that most biologically active compounds in plasma is also reflected in saliva, as the fractions passes easily through the salivary gland basement membrane.[4] The entry of cortisol from the blood into saliva is by transcellular and passive intracellular diffusion.[5]

Interest on saliva as a diagnostic fluid has grown exceptionally in recent years. In this regard salivary samples have the advantage of easy sample collection, non-invasive method, permitting multiple samples and expertise not required.[1] Increased expression of cortisol in saliva reflects stress. Evaluation of this, salivary cortisol may help in the early detection of negative emotional behaviours (Depression, Anxiety and Stress). With the above background this study was taken, to estimate the salivary cortisol levels as a stress

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biomarker in tobacco cigarette smokers.

The study was designed with the following aims and objectives:

1. To estimate salivary cortisol levels in tobacco smokers.
2. To establish the correlation between elevated cortisol levels and stress scale score in tobacco smokers.
3. To ascertain if salivary cortisol levels be used as stress biomarker in tobacco smokers.

MATERIAL AND METHODS

A total of 80 subjects were selected with an age range of 18 to 70 years, inclusive of male gender. The selected patients were clinically examined for their purpose of visit and detailed case history was taken. The procedures required for the study was explained to the patients and they were subjected to questionnaire related to the study. The stress evaluation was assessed using depression anxiety stress scale (DASS). Written informed consent was taken from the subjects for the procedure to be carried out on them. The selected subjects were divided into 2 groups: Group I (Control group) consisted of 40 male subjects of healthy never smokers, Group II (Study group) consisted of 40 male subjects of cigarette smoking with the onset of minimum 6 months and above.

Inclusion criteria

Healthy Subjects with history of cigarette smoking with onset of minimum 6 months and above and using more than 5 cigarettes per day.

Exclusion criteria

Subjects with history of cigarette smoking less than 6 months, Subjects who had quit cigarette smoking, Subjects who were on treatment for psychological disorders, Subjects with passive smoking, Subjects with habit of alcohol consumption, Subjects with disorders of pituitary and adrenal glands and Subjects using other additives with cigarettes were excluded from the study

Method of collection of samples: The salivary samples were collected from the study subjects to estimate the salivary cortisol levels.

Sampling of Saliva: The selected patients were asked to rinse the mouth thoroughly with water. The patients were asked not to eat, drink, and brush teeth for at least 30 minutes prior to saliva collection. Saliva was collected in the morning hours to avoid diurnal variations (8.00 to 9.00 am). Approximately 2ml of unstimulated whole saliva was collected from each subject in a sterile container by spitting method. The container was labelled and transported in an ice pack container to the lab where it will be centrifuged at 3000rpm for 15 minutes to get the supernatant of saliva. The salivary cortisol level was estimated using Chemiluminescent Immunoassay (CLIA).

STATISTICAL ANALYSIS

Independent Student's T-test was used to compare the distribution of age, durationof smoking and number of cigarettes used. Pearson correlation co-efficient was used for comparison of salivary cortisol and stress score.

RESULTS

A total of 80 subjects were selected with an age range of 18 to 70 years, inclusive of male gender.

Age distribution in total sample observations : In Group I, out of 40 (100%) patients, maximum number of patients 17(42.5%), were observed between 21 – 30 years of age. In group II, out of 40(100%) patients, maximum number of patients 16(40%), were observed in the age group between 31-40 years**. (Table1)**

Group II - Distribution of patients with duration of smoking: In group II, out of 40 (100%) patients, maximum number of patients 20 (50%), were observed between 11 to 20 years of duration of smoking. (**Table2)**

Group II - Distribution of patients with number of cigarettes used per day: In group II, out of 40 (100%) patients, maximum number of patients 25 (62.5%) with cigarette use less than 10 numbers per day were observed. **(Table3)**

Evaluation and distribution of patients with depression in the total sample using DAS scale: In group I, out of 40(100%) patients, maximum number of patients were observed with mild depression level, 24 (60%) patients. In group II, out of 40(100%) patients, maximum number of patients were observed with mild depression leve1 18(45%) patients followed by 12(30%) patients with moderate depression level. Severe depression levels were observed in 3(7.5%) patients. 1(2.5%) patient was observed in extremely severe depression level category. **(Table4)**

Evaluation and distribution of patients with anxiety in the total sample using DAS scale : In group I, out of 40(100%) patients, maximum number was observed in mild anxiety level 18 (45%) patients. In group II, out of 40(100%) patients, maximum number was observed with severe anxiety leve1 18(45%) patients, followed by 10(25%) patients in moderate anxiety level. 7(17.5%) patients were in mild anxiety level and least number 2(5%) patients were observed in extremely severe anxiety level category. **(Table5)**

Evaluation and distribution of patients with stress in the total sample using DAS scale: In group I, out of 40 (100%) patients, maximum number was observed in moderate stress level 23 (57.5%) patients. In group II, out of 40(100%) patients, maximum number was observed with severe stress leve1 31(77.5%) patients followed by 7(17.5%) patients in moderate stress level. 2 (5%) patients were observed in extremely severe stress level category. No patients were found in mild and normal stress level category.(**Table6)**

Comparison of salivary cortisol levels between group I and group II: The mean cortisol levels in group I was found to be 0.28 and that of group II was 0.60. The observed difference was found to be highly statistically significant with P value 0.000 **(Table7, Graph 1)**

Comparison of stress score between group I and group II: The mean stress score in groupI was 18.7 and group II

Age group	Group I		Group II	
	Number of patients	n%	Number of patients	n%
Less than 20 years	1	2.5	0	
21 – 30 years	17	42.5	11	27.5
31 – 40 years	8	20.0	16	40.0
41 – 50 years	11	27.5	11	27.5
51 – 60 years	3	7.5	2	5.0
Total	40	100	40	100

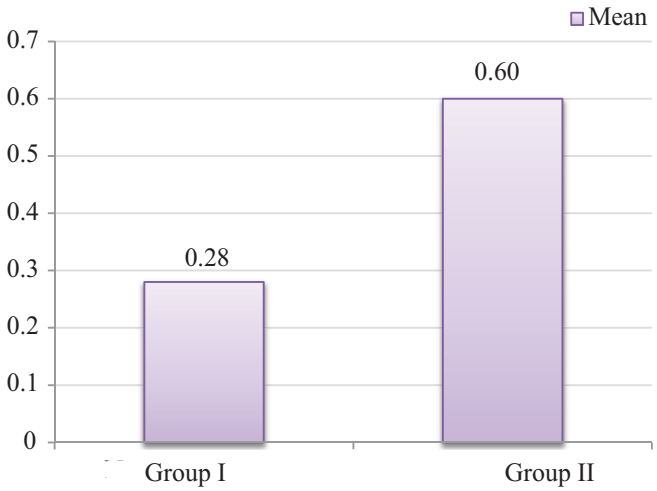
Table-1: Age distribution in total sampleobservations

Duration of smoking in years	Group II	
	Number of smokers	n%
Less than 10yrs	17	42.55%
11 to 20 years	20	50%
21 to 30 years	2	5%
31 to 40years	1	2.5%

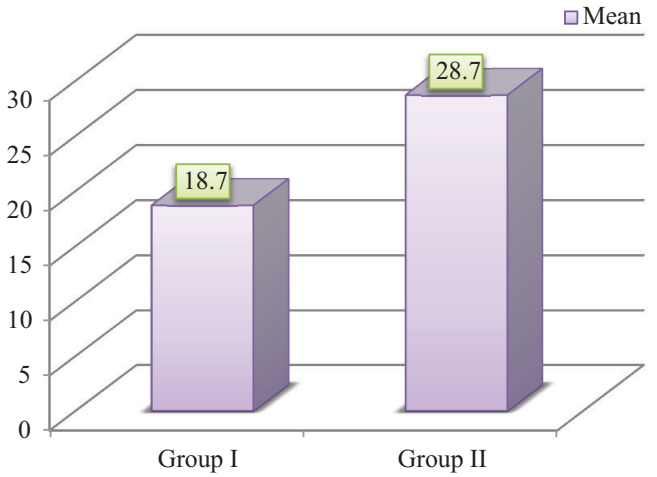
Table-2: Group II - Distribution of patients with duration of smoking

Number of cigarettes per day	Group II	
	Number of patients	n%
Less than 10 cigarettes	25	62.5%
11 to 20 cigarettes	13	32.5%
21 to 30 cigarettes	2	5%

Table-3: Group II - Distribution of patients with number of cigarettes used per day



Graph-1: Comparison of salivary cortisol levels between Group I and Group II



Graph-2: Comparison of stress score between Group I and Group II

Depression level category	Group I		Group II	
	Number of patients	n%	Number of patients	n%
Normal	6	15.0	6	15.0
Mild	24	60.0	18	45.0
Moderate	9	22.5	12	30.0
Severe	1	2.5	3	7.5
Extremely severe	0		1	2.5
Total	40	100	40	100

Table-4: Evaluation and distribution of patients with depression in the total sample using das scale
(Values for evaluation of depression level category (DASS): Normal 0-9, Mild 10-13, Moderate14 - 20, Severe 21-27, Extremely severe 28 +)

Anxiety level category	Group I		Group II	
	Number of patients	n%	Number of patients	n%
Normal	10	25.0	3	4.5
Mild	18	45.0	7	17.5
Moderate	12	30.0	10	25.0
Severe	0		18	45.0
Extremely severe	0		2	5.0
Total	40 (100)	100	40	100

Table-5: Evaluation and distribution of patients with anxiety in the total sample using das scale
(Values for evaluation of anxiety level category (DASS): Normal 0-7, Mild 8-9, Moderate10-14, Severe 15-19, Extremely severe 20+)

Stress level category	Group I n (%)		Group II n (%)	
	Number of patients	n%	Number of patients	n%
Normal	5	12.5	0	0
Mild	12	30.0	0	0
Moderate	23	57.5	7	17.5
Severe	0	0	31	77.5
Extremely severe	0	0	2	5.0
Total	40	100	40	100

Table-6: Evaluation and distribution of patients with stress in the total sample using das scale
(**Values** for evaluation of stress level category (DASS) Normal 0-14, Mild 15-18, Moderate 19-25, Severe 29-33, Extremely severe 34+)

Cortisol (µG/ DL)	Mean	SD	SE of mean	Mean difference	P value
Group I	0.28	0.12	0.02	0.31	0.000, Sig
Group II	0.60	0.1	0.02		

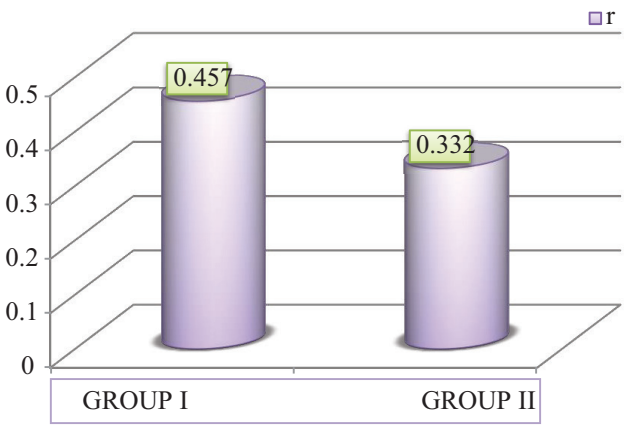
Table-7: Comparison of salivary cortisol levels between group I and group II

Stress score	Mean	SD	SE of mean	Mean difference	P value
Group I	18.7	3.58	0.57	13.642	0.000, Sig
Group II	28.7	2.96	0.47		

Table-8: Comparison of stress score between group I and group II

Salivary cortisol level	Group I		Group II	
	R	P value	R	P value
Stress score	0.457	0.003	0.332	0.037

Table-9: Comparison of salivary cortisol levels and stress score between group I and group II



Graph-3: Comparison of salivary cortisol levels and stress score between Group I and Group II

was 28.7. The observed difference was found to be highly statistically significant with P value of 0.000. (**Table 8,Graph 2)**

Comparison of salivary cortisol levels and stress score between group I and group II: The correlation coefficient of salivary cortisol levels and stress score observed was 0.457 in group I and 0.332 in group II. The observed difference between them was found to be statistically significant. (**Table 9, Graph 3)**

DISCUSSION

Cortisol is the major glucocorticoid in humans and is called as the stress hormone. [1,6] An increased cortisol level in the body reflects HPA axis adaption to stress. The evaluation of salivary cortisol would help in the detection and management of depression, anxiety and stress .Cortisol is routinely used as a stress biomarker. Human saliva is a unique and dynamic diagnostic medium, which mirrors our body's health and is a perfect medium to be explored for assessing biological markers in health and disease.

Age distribution in total sample observations : In group II, maximum number of subjects were observed in the age group between 31-40 years. This is in accordance with studies conducted by Neves et al[7] and Laura Carim et al.[10] Contrary results were observed in studies conducted by Fatima Martinez Slompet [9] al and Mark Hamer et al[8]. This age range could be due to the following reason: Daily stressors are prevalent during midlife.

At this age, responsibility sets in, more stress develops and increased smoking is observed, as smoking may be considered as an important palliative coping style in dealing with stress.[4] Cigarette smoking in younger age group can be attributed to social status, easier availability of cigarettes, affordable cost and peer pressure.

Group II - Distribution of patients with duration of smoking: In group II, maximum number of patients were observed between 11 to 20 years of duration of smoking. This is similar with studies conducted by Neves et al[7] and Zuleya et al.[11] The increased duration of smoking may be

attributed to addictive nature of nicotine to the smokers.
Nicotine increases synaptic neurotransmission in the Central nervous system, particularly of dopamine, which is involved in the rewarding and reinforcing effects of nicotine and plays a key role in addictive properties of tobacco.[12]

Group II - Distribution of patients with number of cigarettes used per day: In group II, maximum number of smokers were found to be using less than 10 cigarettes per day. This is in accordance with the study conducted by Badrick et al[2]. Contrary results were obtained in the study conducted by Laura Carim et al[10], Nevus et al[7] and Miranda et al[13]. People habitually smoke cigarettes in specific situations such as after a meal, with a cup of coffee or an alcoholic drink, or with friends who smoke. Even unpleasant moods can become conditioned cues for smoking. For example, a smoker may learn that not having a cigarette provokes irritability (a common symptom of the nicotine abstinence syndrome) and smoking a cigarette provides relief[14,15]. Shafer postulated that stress decreases the action of nicotine, thereby resulting in increased nicotine self–administration to reach the levels of activity that occur in non-stressful circumstances. Also, stress decreases the availability of nicotine, precipitating withdrawal and resulting in subsequent increased tobacco use.

Evaluation and distribution of patients with depression in the total sample using DAS scale: In Group I, most patients were observed in mild and moderate depression level category. Normally, daily stressors are prevalent during mid-life, this could be transient and can go unnoticed because the observed cortisol levels were within normal range.
In group II, most smokers were observed with mild and moderate depression level category. This was in accordance with studies conducted by Fatima Martinez Slomp et al[9] and Talukdar et al[3], Badrick et al[2]. Increased risk of depression in smokers, may be attributed to the fact that, Smoking causes decreased 5HT (serotonin) function, as there is increased production of cytokines due to smoking which alters the serotonin metabolism causing increased symptoms of depression in smokers.[16] Cigarette smokers frequently describe the anxiolytic and antidepressant effects of smoking.[17]
Depression is an absolute or relative deficiency of Monoamines (MAO). MAO is an enzyme responsible for degrading the neurotransmitters nor-epinephrine, serotonin and dopamine from brain which contributes to depression as these neurotransmitters plays a role in mood modulation, increased levels of arousal and cognitive enhancement.
Smoking induces down regulation of Mono Amino Oxidase (MAO) activity. Therefore, it is suggested that MAO inhibition by cigarette smoke ameliorate symptoms of depression in smokers.[18,19] Stressful life experiences may, further increase the propensity for smoking in both depressed as well as non-depressed condition.

Evaluation and distribution of patients with anxiety in the total sample using DAS scale : In Group I, most patients were observed with mild anxiety and moderate anxiety level category. This could be the situational anxiety of the hospital set up, which is transient. In group II, most smokers were observed with severe anxiety level category. This was in accordance with studies conducted by Pavani Reddy et al[1], Fatima Martinez Slomp et al [9] and Talukdar et al[3] where they found the same results.
Various mechanisms by which smoking increases the risk of anxiety is as follows: Serotonin (5-HT) neurotransmission dysfunction can be linked to many psychiatric and behavioural conditions, particularly anxiety and mood disorders. Smoking causes decreased 5-HT function and this reduction in brain serotonin leads to depression and increased anxiety symptoms.The increased levels of pro-inflammatory cytokines are a significant risk factor for developing anxiety and depression. Depression brings about disturbances in important stress systems of human body i.e., HPA axis and autonomic nervous system which in turn might stimulate the production of cytokines. Also, increased inflammatory cytokines are produced in response to smoking. This pro-inflammatory cytokines can influence the serotonin metabolism by activating indoleamine 2, 3- dioxygenase to convert tryptophan to typtoph- catoline including quonolic acid, an in-lieu of serotonin, which is associated with increased anxiety and depressive symptoms. Depression and anxiety are related to lowered plasma concentration of antioxidants. Smoking reduces antioxidant levels and increases oxidative stress of the body. Free radicals are highly concentrated components of cigarette smoke which stimulates the production of CMI cytokines and proinflammatory cytokines which on increased concentration can influence the serotonin metabolism and is associated with depression and anxiety disorders. Also, reactive oxygen species (ROS) from tobacco smoke interact in a bidirectional fashion with proinflammatory cytokine pathway and leads to down regulated production of nitric oxide synthase due to which enhanced anxiety results.[16,18,19]

Evaluation and distribution of patients with stress in the total sample using DAS scale:
In Group I, maximum number of patients were with mild and moderate stress. In Group II, most smokers were observed with severe stress level category.This was in accordance with studies conducted by Laura Carim et al [57]and Mathew. C. Morris et .al [61]. Mark hammer et al[8], disclosed that cortisol reactivity, (an index of hypothalamopituitary-adrenal function) is one of the possible mechanisms through which psychosocial stress may influence the risk of hypertension.. Tobacco smoking (and nicotine) enhances cognitive functions which overcome the deleterious effects of stress on cognition. This enhancement of cognitive function is brought about by the stimulation of nicotine acetyl choline receptors in brain by nicotine, which results in the release of a variety of neurotransmitters in the brain, most importantly dopamine where dopamine appears to be critical in drug-induced reward and is responsible for the mood elevation and apparent improvement in cognitive function in smokers.[14]

Comparison of salivary cortisol levels between group I and group II: The mean cortisol levels in group I was found to be 0.28 and that of group II was 0.60. The observed difference was found to be highly statistically significant with P value 0.000

The mean salivary cortisol level observed in group II was high when compared to group I. This was in accordance with studies conducted by Badrick et al[2], Talukdar et al[3] and Kirschbaum et al[53] where the authors found similar results. This was contrary to a study by Zuleyha et al[11] and Nao Suzuki et al[21].

According to Talukdar et al [3], salivary cortisol levels in smokers may provide a useful indicator for exploring the mechanisms by which nicotine-stress interactions are mediated and will also help to resolve whether the effect of chronic smoking on salivary cortisol may potentially have large consequences on downstream endocrine function.

Smoking cigarettes increase peak nicotine levels in the brain, followed by an activation of HPA axis which leads to increased secretion of cortisol from the adrenal glands which is then taken to different sites for function through blood. The increased cortisol in blood is reflected in saliva through, transcellular, passive and intracellular diffusion. Active transport or extra cellular ultrafiltration within the salivary gland acini and to saliva and thus presence of salivary cortisol in saliva.[22] The presence of increase in salivary cortisol indicates stress.

Comparison of stress score between group I and group II: The mean stress score was found to be more in Group II when compared to Group I. The observed difference was found to be highly statistically significant with P value of 0.000. This was in accordance with studies conducted by Talukdar et al[3] and Laura Carim et al[10]. Smokers yield to smoking habit during stress, or they develop an urge to smoke assuming that they can be free of stress at that part of time

Comparison of salivary cortisol levels and stress score between group I and group II:. The correlation coefficient of salivary cortisol level and stress score observed in group II was high when compared to group I. The observed difference between them was found to be statistically significant. This was in accordance with studies conducted by Miranda et al[13], Robert S Stansky et al[4], Uma Rao et al[23] and Talukdar et al[3]. This mechanism could be a double faced process. Stress indulges in smoking and vice versa. Smoking increases salivary cortisol level through the action of nicotine. Smoking cigarette increases peak nicotine levels in the brain, followed by an activation of HPA axis. Stress is evaluated through presence of increase in salivary cortisol level, and this is elevated in smokers due to the action of nicotine in cigarette smoke[14,22,24].

CONCLUSION

In the present study it was observed that, salivary cortisol levels were increased in cigarette smokers which ascertained the use of salivary cortisol levels as a stress biomarker in tobacco cigarette smokers. Saliva as a diagnostic medium has added expediency of easy, non-invasive sample collection, and is non- stressful to the patient. Estimation of salivary cortisol can be used as a stress biomarker in tobacco cigarette smokers and can permit its use in large scale screening programmes. Moreover, the implication of salivary cortisol levels on these psychological disorders may help in their early diagnosis and better management. This can further contribute in counselling and cessation of the tobacco habit thus preventing its impact on other systems of the body.

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