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A Study on Comparison of Salivary Cortisol Circadian Rhythm in Periodontal Diseases with External Stressors and Clinical Parameters

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ABSTRACT

It is found that salivary cortisol values follow the same pattern as the serum cortisol. They are found to have high correlation with some parameters showing disturbances in HPA –axis. Cortisol levels rise due to circadian influences as well as perturbations in the organism's environment (i.e., stressors) that make it possible to detect. The following study was done to study the effect of HPA axis in the salivary cortisol and daily variation. It was also done to study the circadian rhythm of the hormone in patients with periodontal diseases and the correlation of salivary cortisol with loss of attachment in periodontium. The study was planned for a population with periodontitis and control subjects. All participants, aged 20-50 years, were examined at the clinics of Ajman University (Al-Fujairah). They were informed to provide three samples that were stored at 3-5 degrees and submitted next day. Samples were frozen at (– 20°c) and analyzed in COBAS e 411 by ECLIA (Electro-chemiluminescence) method. The highest mean value of salivary cortisol was 30 minutes after waking up for both groups, but was higher in the control group. BMI, S-OHI and HAM-A had the highest correlation in both groups. No correlations were found with periodontal loss of attachment and pocket depth. Cortisol gap showed a more positive trend in the periodontitis group. The circadian rhythm is established in salivary samples and follows the same pattern in serum. Cortisol gap can be used instead of cortisol awakening response as a diagnostic value in defining periodontal diseases and its severity.

Key words: Cortisol, Periodontitis, HPA axis, Electro chemiluminescence.

INTRODUCTION

The three endocrine glands that they form an axis called HPA axis is constituted by hypothalamus, pituitary and the adrenal glands. These three glands interact each other and have influences on each other. This neuro-endocrine system is responsible for the regulation of stress and control the body metabolisms such as emotion, immune system, digestion, sexuality, and energy preservation[10].

Pituitary gland connects superiorly with the hypothalamus via infundibulum which is funnel shape. It divides into two parts or lobes one of them glandular and the other is neural tissue. The anterior

part is the glandular which has the responsibility of manufacturing and releasing hormones. The posterior part and infundibulum form a complex known as neurohypophysis, this complex has the duty to store the hormones. Hypophyseal branches of the internal carotid arteries supplies the blood to the pituitary gland while the drainage via veins into the dural sinuses[4].

Posterior pituitary and the hypothalamus are related by the formation of oxytocin and ADH by the hypothalamic neurons. These hormones are transported via hypothalamic-hypophyseal tract to the posterior lobe of pituitary gland.

Adrenal glands are pyramid-shaped and lie above the kidney. They consist of two components which are adrenal medulla and adrenal cortex. Each of them releases a specific type of hormones[4].

Essentials for the function of the HPA axis

The inhibition of the hypothalamus and the pituitary glands is by the negative feedback from the cortisol that is produced in the adrenal cortex. This results in reduction of CRH and vasopressin, and also minimize the cleavage of proopio-melanocortin into ACTH and β-endorphins[1].

Sympathateic stimulation and the local effect of cortisol can induce the secretion of epinephrine and norepinephrine which both lead to positive feedback to the pituitary gland that increases the breakdown of POMCs into ACTH and β-endorphins[10].

The pathophysiology of HPA axis

Stress, illness, physical activity, blood level of cortisol and sleep\wake cycle which known also as circadian rhythm are the main factors that result in releasing of CRH(corticotrophin releasing hormone). In normal people after wakening, the cortisol level rapidly increases. After 30-45 minutes it reaches the peak, while through the day it decreases, and increases again at afternoon period. After that it decreases in late evening. If there is abnormality like insomnia, burnout or chronic fatigue the circadian cortisol cycle will be flattened[2].

The HPA axis regulates physical and psychosocial effects that leads to the adaptation of its environment and maintain the situations. It plays a main role in regulation of systems such as metabolic system, immune, reproductive, central nervous system and cardiovascular system[2].

Because of the stress, the releasing of cortisol will be more which leads to increase the glucose that mediate fighting. This procedure decreases the highly demanding metabolic processes of immune system[2].

On the other hand, the high level of glucocorticoids that result from severe stress will lead to damage hippocampus of human body. As a conscious it reduces the memory resources that help the individual to react against stress[2].

Cortisol is a lipophilic steroid with low molecular weight. It is synthesized and released into the blood stream, following ACTH binding to membrane receptors on cells of the adrenal cortex. Up to 95% of the secreted cortisol will be bound to large proteins (CBG, albumin) and carried throughout the body in the blood. The vast majority of cortisol actions rely on binding to its cytosolic mineralocorticoid and glucocorticoid receptors, so only the small fraction of unbound, i.e., free cortisol is thought to be biologically active. Unbound cortisol enters cells by passive diffusion, Due to its low molecular weight and lipophilic nature, which makes it feasible to measure the free cortisol fraction in all bodily fluids. While the assessment of cortisol in sweat or tears is only of theoretical importance and urinary cortisol of decreasing interest, salivary cortisol has become an invaluable tool for both clinicians and basic scientists. A number of significant advantages over the assessment of cortisol in blood have resulted in a steadily increasing interest in salivary cortisol[3].

The concentration of cortisol in saliva accurately reflect the level in blood when compared to the amount of unbound cortisol in serum or plasma samples however, the correlation between the total cortisol levels in blood and salivary cortisol is usually weaker due to different amounts of CBG found in blood[3].

The salivary cortisol levels don't depend on saliva flow rate relying on studies looking at salivary cortisol levels obtained under minimal and maximal flow rate. The level of salivary cortisol is influenced by drugs such as prednisone, dexamethasone and other steroids administered orally or IV While prednisone usually cross reacts with the antiserum used for assaying cortisol (leading to false high values), dexamethasone will significantly suppress the HPA axis (resulting in low cortisol levels)[3].

Periodontal disease may aggravate with stress and depression. The individuals who has stress will have less ability for brushing their teeth & there will be an association of loss of attachment >5mm. Addressing psychological factors, such as depression, may be an important part of periodontal preventive maintenance[6].

MATERIALS AND METHODS

The study was conducted after getting the ethical clearance from Ajman university of science and technology.All the patients were given the questionnaires to assess their anxiety level and clinical examination form was filled along with distribution of saliva collecting vials. Donors were asked to collect whole saliva by tilting the head forward, allowing the saliva to pool on the floor of the mouth, and then passing the saliva through the saliva collection aid into a polypro-pylene vial supplied by the CABRI labs UAE. The subjects were given instruction not to eat or drink for 1 hour before the sample collection is planned. The amount of saliva collected is 2 ml. All the subjects are motivated to participate in the study by signing the patient participation form. The saliva is collected at 3 timings on the same day to find out the pattern of circadian rhythm, the first one immediately after waking up, second one 30 minutes after the first one and the third one 1 hour before sleeping. The collected samples were stored in home refrigerator at 3-5 degrees and handed over to the Ajman University next day morning. The Samples were transported to biotechnology lab to be frozen under-20degree Celsius. The patients were divided in to 2 groups. Group A (control group) without any clinical evidence of gingivitis or periodontal disease and group B with clinically and radio graphically established cases of chronic adult periodontitis. Sample size selected was 12 subjects in each group with 3 different readings for each patient and investigators were expected to collect 36 samples in each group to be tested. The salivary cortisol would be evaluated with electro-chemiluminescence immunoassay method (ECLIA) using Cobas e- 411 autoanalyser by ROCHE USA.

Exclusion criteria

1. Patients with any systemic disease other than the ones investigated for.
2. Patients who are pregnant/likely to be pregnant.
3. Patients who are having any adverse habits (Tobacco (smoking- chewing), Bruxism, Alcohol) which can affect the said study.
4. Patients who are taking any sort of corticosteroids in any formulations.

Inclusion criteria

All the patients will be examined from university student clinics in the age range of 15-70 yrs.

Group A

Control group – healthy individuals without any habits and systemic diseases.

Group B

Patients who are diagnosed with chronic adult periodontitis.

Table 1: Mean and standard deviation of both groups

group	N	Mean	Std. Deviation
a Perio.	10	17.6190	9.26061
control	11	21.2582	11.42968
b Perio.	10	25.4710	11.24774
control	11	31.2727	17.08145
c Perio.	10	8.5180	4.13991
control	11	20.3836	16.98513

*a= salivary cortisol level immediately after waking up

* b= salivary cortisol level after 30min of waking up

*c= salivary cortisol level before 1hr of sleeping

In chart 1 the percentage of the three categories (fair, Good and Poor) are presented for each group for Simplified oral hygiene index. In the control group the highest percentage was for the fair group while the lowest percentage was for the good, while in the Periodontitis group there was an equal percentage for the Poor and the good category while the fair category was 60%.

Chart 2 shows that there is correlation between the time and the salivary cortisol level during the day for both control and periodontitis groups. Sample after 30 minutes of waking up it shows that there is increase in the salivary cortisol level compared to salivary cortisol level immediately after waking up. The level of cortisol in control patients showed comparable value in the morning and in the evening but the value for chronic

Table 2: T- test paired samples for the periodontitis group

		N	Correlation	Sig.
Pair 1	BMI & a	10	.731	.016*
Pair 2	BMI & b	10	.730	.017*
Pair 3	BMI & c	10	.230	.523
Pair 4	PBI & a	10	.157	.665
Pair 5	PBI & b	10	-.048	.895
Pair 6	PBI & c	10	.190	.599
Pair 7	Waist circumference & a	10	.414	.235
Pair 8	Waist circumference & b	10	.584	.076
Pair 9	Waist circumference & c	10	.563	.090
Pair 10	Highest value of periodontal pocket in mm & a	9	-.332	.382
Pair 11	Highest value of periodontal pocket in mm & b	9	-.247	.521
Pair 12	Highest value of periodontal pocket in mm & c	9	-.330	.386
Pair 13	Highest value of loss of attachment in mm & a	10	.213	.554
Pair 14	Highest value of loss of attachment in mm & b	10	.241	.503
Pair 15	Highest value of loss of attachment in mm & c	10	-.030	.935

*a= salivary cortisol level immediately after waking up.

* b= salivary cortisol level after 30min of waking up.

*c= salivary cortisol level before 1hr of sleeping.

Table 3: T- test paired samples for the control group

		N	Correlation	Sig.
Pair 1	BMI & a	11	-.182	.592
Pair 2	BMI & b	11	-.409	.212
Pair 3	BMI & c	11	-.166	.626
Pair 4	PBI & a	11	-.503	.115
Pair 5	PBI & b	11	-.533	.092
Pair 6	PBI & c	11	-.234	.489
Pair 7	Waist circumference & a	11	-.113	.742
Pair 8	Waist circumference & b	11	-.451	.163
Pair 9	Waist circumference & c	11	-.255	.448

*a= salivary cortisol level immediately after waking up.

* b= salivary cortisol level after 30min of waking up.

*c= salivary cortisol level before 1hr of sleeping.

periodontitis patients showed a severe drop in the night.

Comparing the salivary cortisol level of the control patients immediately after waking up to the patients with periodontitis, the difference between both Mean is equal to (3.64). After 30 minutes of waking up, the difference between both Mean of salivary cortisol level in the control and the periodontitis groups is (5.8) and it is (11) at 1 hour before sleeping. We noticed that in control patients the mean of salivary cortisol level is higher in all timing comparing to the periodontitis patients group.

There is significant correlation between the values of cortisol soon after waking up (sample a) and sample b with BMI. There is no other correlation of any 3 cortisol values with any clinical parameters.

From the table 4, we found out that there is no correlation between any of the parameters in control patients with the salivary cortisol level immediately after waking up (a). Moreover; The

Table 4: Correlations between the parameters and the salivary cortisol level in control patients

S. No.		HAMA-A	Waist Circum.	Sleeping pattern	PBI	SOHI	BMI
1	Pearson Correlation	.794**	-.113	.569	-.503	-.227	-.182
	Sig. (2-tailed)	.004	.742	.068	.115	.503	.592
	N	11	11	11	11	11	11
2	Pearson Correlation	.484	-.451	.614*	-.533	-.618*	-.409
	Sig. (2-tailed)	.131	.163	.045	.092	.043	.212
	N	11	11	11	11	11	11
3	Pearson Correlation	.666*	-.255	.285	-.234	-.203	-.166
	Sig. (2-tailed)	.025	.448	.396	.489	.548	.626
	N	11	11	11	11	11	11

*a= salivary cortisol level immediately after waking up. * b= salivary cortisol level after 30min of waking up.
*c= salivary cortisol level before 1hr of sleeping.

Table 5: Correlations between the parameters and the salivary cortisol level in chronic adult periodontitis patients

S. No		HAMA-A	Waist Circum.	Sleepin g pattern	Highest v. of attachment loss	PBI	SOHI	BMI
A	Pearson Correlation	.642*	.414	.131	.213	.157	.667*	.731*
	Sig. (2-tailed)	.046	.235	.718	.554	.665	.035	.016
	N	10	10	10	10	10	10	10
B	Pearson Correlation	.416	.584	-.121	.241	-.048	.922**	.730*
	Sig. (2-tailed)	.231	.076	.739	.503	.895	.000	.017
	N	10	10	10	10	10	10	10
C	Pearson Correlation	.488	.563	.178	-.030	.190	.537	.230
	Sig. (2-tailed)	.153	.090	.623	.935	.599	.109	.523
	N	10	10	10	10	10	10	10

*a= salivary cortisol level immediately after waking up. *b= salivary cortisol level after 30min of waking up.
*c= salivary cortisol level before 1hr of sleeping.

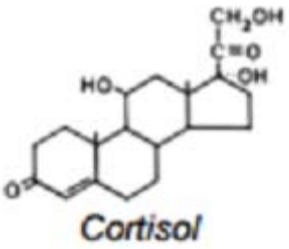

Fig. 1: Cortisol chemical formula

Fig. 2: Collecting the samples at 3 different times

Fig. 3: Centrifuge the salivary samples

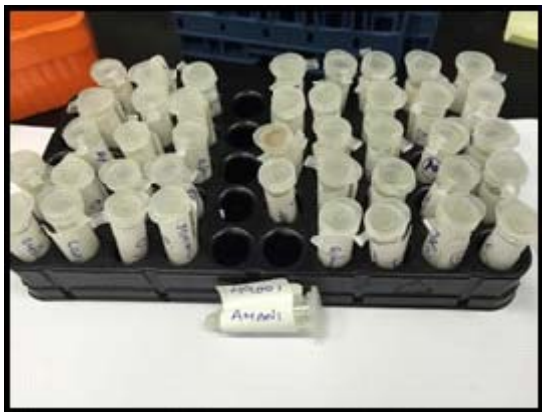

Fig. 4: Arrangement of salivary samples for incubation

Fig. 5: Incubation of samples under 16 centigrade

Fig. 6: Cobas e- 411 autoanalyser by ROCHE USA

salivary cortisol level after 30min of waking up has correlation with the sleeping pattern and the oral hygiene index of the control group (b). Furthermore; there is correlation between the anxiety scales of the control group with the salivary cortisol level at 1 hour before sleeping(c).

From the table 5, we found out that there is correlation between the anxiety scale, oral hygiene index and body mass index in chronic adult periodontitis patients with the salivary cortisol level immediately after waking up (a). Also, the salivary cortisol level after 30min of waking up has

Fig. 7: Running the samples in the machine.

Fig. 8: CORT reagent

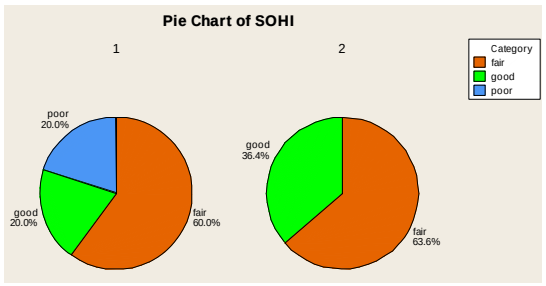

Chart 1: Pie chart of SOHI (simplified oral hygiene index) for both periodontitis and control groups

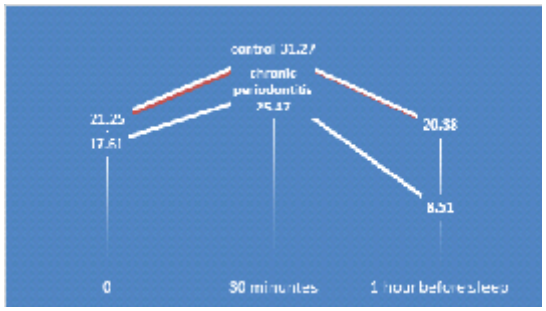

Chart 2:

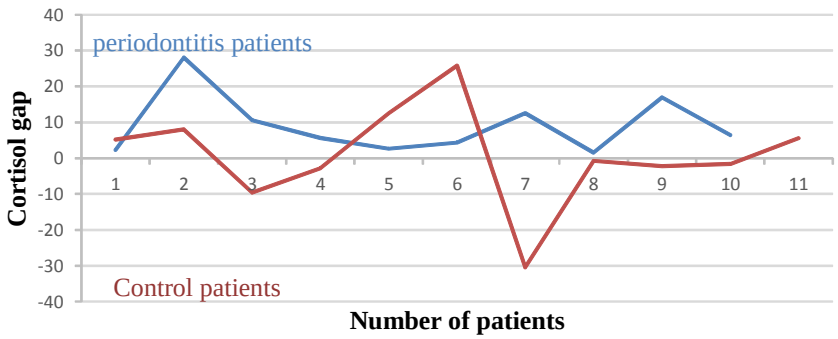

Chart 3: Chart shows the relation between the number of patients and the cortisol gap on both groups

correlation with the body mass index of the periodontitis group (b). But, there is no correlation of any of the parameters with the salivary cortisol level at 1 hour before sleeping (c).

The difference between the salivary cortisol level sample a (soon after waking up) and the salivary cortisol level of sample c (1 hour before sleeping) showed that it is higher in periodontitis patients compared to control patients. Moreover; the values are more dispersed in control compared to periodontitis group.

DISCUSSION

This study has showed clearly a circadian rhythm in the samples in both the groups. All patients have been informed to obtain three samples (to spit without eating and drinking anything), first one immediately after waking up, the second one, 30 minutes after waking up, and the last one in the evening one hour before sleeping. It clearly showed that a circadian rhythm with cortisol awakening response was observed, with the second sample cortisol levels noticeably greater than the others (peak value) followed by a plateau in the evening.

Our study showed that there is statistically significant association between the body mass index (BMI) and the salivary cortisol level immediately after waking up and the salivary cortisol level after 30 min of waking up in periodontitis patients.

C.G. Tornhage and G.Alfven (2006), did a study which says that the increase of the morning salivary cortisol level and total cortisol concentration is negatively correlated with the body mass index[4]. Laura Manenschijn Rulanda G. P. M. Van kruysbergen(2004), conducted a study that showed results comparative to our results by proving that salivary cortisol level elevation has relation with BMI[(9)]. In another study by S wallerius, R. Rosmond (2014), showed that rise in the morning salivary cortisol is associated with abdominal obesity [(10)]. In our study it showed that there is no correlation between sleep pattern & salivary cortisol level. On the other hand, Jutta backhaus (2004), proved that there was decrease on the morning cortisol because of sleeping disturbances[7].

In control group, we noticed that there is a strong correlation between the salivary cortisol level and the oral hygiene index 30 minutes after waking up, but in periodontitis group there is a correlation between salivary cortisol level and the oral hygiene index immediately after waking up.

J. B. Hilgert (2006), conducted a study in chronic periodontitis and found that women with stress will have more plaque accumulation & increased levels of IL-6 and cortisol in gingival curricular fluid than the normal control[6].

In control patients, HAMA-A(Hamilton's anxiety scale) has correlation with the salivary cortisol level 1 hour before sleeping, while in the periodontitis group we noticed that there is correlation between HAMA-A and the salivary cortisol level immediately after waking up.

Kav vedhara (2003), did a study that there was non-linear relation between cortisol level and the time of the day, but the extend of non-linearity was dependent upon levels of stress and anxiety[(8)].Van Eck (1996), did a study that focused on stress and anxiety that leads on increasing of the cortisol secretion depending on whether the event was still ongoing and on the frequency[(12)].

Arash azizi (2013) studied the mean of the hospital anxiety and depression scale(HAD scale) with salivary cortisol level and was found to be 14.07 ± 6.10 in periodontitis group and 11.27 ± 4.24 in control group, and there is positive correlation between the anxiety scale and periodontitis[1].

The difference between the salivary cortisol level on the early morning and the salivary cortisol level at evening 1 hour before sleeping is termed as cortisol gap. This is a new parameter investigated for as it showed some significance. It showed that it is higher in periodontitis patients comparing to control patients. But in another study done by Arash azizi1 (2013), showed that the salivary cortisol mean is higher on periodontitis (56.7%) than the control (13.3%)[1].

In our study, we noticed that there is no correlation between the loss of attachment, the periodontal pocket depth, periodontal bleeding index (PBI) and the salivary cortisol level. However in a study conducted by J. B. Hilgert (2006), it was noticed that there is positive correlation between the cortisol level and the loss of attachment level (> = 4 mm) and with periodontal probing depth > = 4 mm(6).

In future studies as investigators, we can recommend using cortisol gap as a parameter to study the effects along with cortisol awakening response as it shows a more reliable response compared to the day light variation of cortisol release. The advantage being this cortisol gap (the difference in the values between morning level of cortisol soon after waking up and the one hour before sleep is less influenced by the chemicals from the pineal gland and external stressors.

CONCLUSIONS

In periodontitis patients, there is statistical significance observed between the body mass index (BMI) and the salivary cortisol level immediately after waking up (sample a)and 30 min after waking up(sample b). It was also observed that there are no statistical differences found between any of other parameters in periodontitis patients. But in control patients, there are no statistical significances existing between any of the parameters with the salivary cortisol level. But there is a strong correlation between sleeping pattern and salivary cortisol level 30 minutes after waking up. It has shown that in control group there is a correlation between patients' oral hygiene index (S- OHI) and salivary cortisol level 30 minutes after waking up. HAMA-A / anxiety level index has a strong correlation existing with the salivary cortisol level 1 hour (sample c) before sleeping.

There is no severe drop in the cortisol values during night in control patients compared with chronic adult periodontitis where the cortisol gap(difference between values of sample a and sample c) was found to be higher in value.

REFERENCES

1. Arash azizi, Fatemeh sarlati., Salivary cortisol levels and moderate to severe periodontitis, *Research Dental Science* 2013; **11**(1): 32-37.
2. C. Tsigos, G.P. Chrousos., Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress, *Journal of Psychosomatic Research* 2002; **53**(25): 865-871
3. Clemens Kirschbaum, Dirk H. Hellhammer., Salivary Cortisol, *Encyclopedia of stress*, 2000; **3**(7): 379-383
4. C.G. Tornhage and G.Alfven.,Diurnal Salivary Cortisol Concentration in School-aged Children, *Journal of Pediatric Endocrinology and Metabolism* 2006; **19**(6): 843–854.
5. Elaine N. Katja Hoehn., Human Anatomy and Physiology, eighth edition, Benjamin Cummings, 2010.
6. J.B. Hilgert, F.N. Hugo, D.R. Bandeira, M.C. Bozzetti., Stress, Cortisol, and Periodontitis, *Journal of periodontology* 2006; **85**(4): 324-328
7. Jutta Backhaus, Klaus Junghanns., Sleep disturbances are correlated with decreased morning awakening salivary cortisol, *Inernational socity of Psychoneuroendocrinology* 2004; **29**(9): 1184–1191.
8. Kav Vedharaa, Jeremy Milesc, Paul Bennettd Sue Plummer., An investigation into the relationship between salivary cortisol, stress, anxiety and depression, *Biological Psychology* 2003; **62**(2): 89-96.
9. Laura Manenschijn, Rulanda G. P. M. van Kruysbergen., Shift Work at Young Age Is Associated with Elevated Long-Term Cortisol Levels and Body Mass Index, *Journal of Pediatric Endocrinology and Metabolism* 2011; **96**(11): 2-5.
10. S. Wallerius, R. Rosmond, T. Ljung, G. Holm., Rise in morning saliva cortisol is associated with abdominal obesity in men, *Journal of endocrinological investigation* 2003; **26**(7): 616-619.

11. Sandra Marcia. Cristiane Von Werne. Camila Maria. Mario Juruena., Early life stress, HPA axis, and depression, *Psychology & Neuroscience* 2011; **4**(2): 229-234.

12. van Eck, Marleen, Hans.,The Effects of Perceived Stress, Traits, Mood States, and Stressful Daily Events on Salivary Cortisol, *Psychosomatic Medicine* 1996; 58(5): 447-458.