ORIGINAL RESEARCH

A Clinical Study on the Circadian Rhythm of Salivary Cortisol on Aggressive Periodontitis and Its Correlation with Clinical Parameters using Electrochemiluminescence Immunoassay Method

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Abstract

Aim: Periodontal pathologies are gaining importance as there is a clear indication of bi-way control on general homeostasis of an individual. The study of HPA axis in various diseases has proved that there is evident vulnerability existing for any organism when the Cortisol diurnal rhythm is altered. The aim was to compare the diurnal rhythm of salivary cortisol in aggressive periodontitis with control patients. This study also compared various parameters like body mass index (BMI), waist circumference, Hamilton anxiety scale, OHI-S, clinical attachment loss in aggressive periodontitis.

Materials and methods: 30 control patients were compared against 30 aggressive periodontitis patients in Salivary cortisol diurnal rhythm. It was estimated using the electrochemiluminescence (ECL) method on a 3 point analysis—Soon after waking up, 30 minutes after waking up, 1 hour before sleep to see the diurnal variation in aggressive periodontitis patients. The samples were transferred to CABRI labs to be frozen to –20°C. The analysis was done using Cobas e-411 autoanalyzer by Roche, USA.

Results: The average cortisol in aggressive patients was found to be higher compared to control patients and was found to be statistically significant with a p value of 0.012. Control group is moderately skewed left (negative skewness graph) while the aggressive p periodontitis patients showed moderately skewed right (+ve skewness graph).

Conclusion: The cortisol awakening response seen in control patients is not observed in aggressive periodontitis. Instead of giving a surge, the cortisol showed a dip in the first 30 minutes followed by a gradual increase in aggressive periodontitis instead of decline as observed in normal patients.

Clinical significance: The study will focus on the importance of cortisol circadian rhythm on periodontal health allowing the microorganism to create an environment of dysbiosis.

Keywords: Aggressive periodontitis, Circadian rhythm, Electrochemiluminescence, Salivary cortisol.

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Introduction

Periodontitis is multifactorial disease often started by host triggers which ultimately results in infection and destruction of the periodontium. Periodontal diseases are characterized by the response that the host is rising against the bacterial challenge or the biofilm. The concept of pathobiont and dysbiosis is relevant as pathobiont relates to a harmless symbiont which can become pathogen under certain environmental conditions. The term dysbiosis is related to an imbalance in the relative abundance of microbial species that are related to pathology and is within an ecosystem. There are of two types of periodontal diseases which are classified as chronic and aggressive types. An abundance of plaque and calculus often characterizes chronic periodontitis. Aggressive periodontitis is identified as symptoms are not very obvious, but there is a rapid progression of disease with a severe bone loss not correlating to the amount of biofilm or calculus present. In aggressive forms often there is no clear correlation with the extent of destruction and amount of biomarkers that are seen. The major etiological agent being Aggregatibacteractinomycetemcomitans releases immunosuppressive factors and toxins, like leucotoxin, endotoxin, and cytotoxins, which aim to modify the host response in favor of its continued proliferation and invasion.[1] Localized forms of PD are associated with Aggregatibacter actinomycetemcomitans, while chronic generalized forms of the disease are related to several bacteria including Porphyromonas gingivalis, Tannerella forsythia, Treponemadenticola, and others. The microbial diversity of the oral cavity is immense, and it is clear that the host response during periodontal disease is complex with innate and adaptive elements driving chronic inflammation and bone loss.[2]

Aggressive is less prevalent when compared to chronic periodontitis. Aggressive periodontitis is further subclassified based on the spread as localized or generalized types. Aggressive periodontitis is relatively infrequent, and the number of studies that

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have been done is less. The distribution frequency of aggressive periodontitis varies between continents, with ethnicity, sex, and age. The aggressive type of disease shows a prevalence rate of around 1–5% in the African population and groups of African descent, 2.6% in African-Americans, 0.5–1.0% in Hispanics in North America, 0.3–2.0% in South America, and 0.2–1.0% in Asia. Among Caucasians, the disease prevalence is 0.1% in northern and in central Europe, 0.5% in southern Europe, and 0.1–0.2% in North America.[3] The distinction between chronic and aggressive is made on the following salient features which include age of onset, a rate of progression, patterns of destruction, clinical signs of inflammation and relative abundance of plaque and calculus. Age is a significant feature in deciding the nature of periodontitis. Generally aggressive is seen more in young patients than chronic periodontitis. Chronic periodontitis is slow progressing whereas aggressive is rapid. The pattern of destruction is one of the major indicators of the type of periodontitis. No definitive pattern is seen in chronic periodontitis with few teeth or whole dentition showing the infection. Most of the permanent teeth are affected in generalized aggressive periodontitis. Aggressive periodontitis is having usually less inflammation with thin b
least in localized type.[4]

Stress and HPA axis

Stress disrupts the balance in the body. There is
stressors to which the body is exposed. Environ
role in the whole process. The hypothalamic-p
a vital role in the stress-mediated response. Th
balance out stress by secretion of molecules tha
the balance in the body. In response to stress,
secretes two important hormones CRF and
the hormones stimulate the anterior pituitary
corticotropins. The secretion of ACTH results in a
glands to release cortical hormones. Cortisol bi
to the mineralocorticoid receptors (MRs) than
receptors (GRs). Because of this difference in b
corticoid receptors help in maintaining relat
levels circulating in the blood during the no
conditions. Only when the cortisol concentra
during a stressful situation it binds to the GRs
thus resulting in activation of the GRs which ter
response. Differences in response to cortisol va
to individual. This is dependent upon the genetic variability which ultimately controls the expression that is seen across the HPA axis.[5,6] Chronic stress triggers a shift in the normal circadian rhythm of cortisol release as well as in stress-induced cortisol levels. Thus, after chronic stress baseline cortisol levels are elevated, the body's cortisol response to acute stress is altered, and it takes longer for stress-induced cortisol levels to return to pre-stress levels.

Cortisol, interleukin-1 beta, and Interleukin-6 have been shown to be markers associated with a periodontal breakdown. Various molecules released during stress directly influence the immune system and affect the production of interleukins thus affecting the immune response. Cortisol can be measured in a variety of body fluids including tears, sweat, urine, and saliva. Salivary cortisol levels have become an important parameter for assessing the functioning of the hypothalamic-pituitary axis. The levels of cortisol vary in saliva. It exhibits a diurnal pattern with cortisol levels moderate after waking up and highest 30 minutes post waking up. The levels are low when 1 hour before sleep measured. Therefore the levels 30 minutes post wake up play an essential role in understanding the difference between normal and patients. This increased levels post waking up is referred to as cortisol awakening response.[7] Several studies suggest that a large or small cortisol awakening response (CARCAR) and shallow or flat slopes, i.e., small declines in cortisol secretion across the day, is associated with poor health and associated outcomes.[8,9]

Bone Loss and Cortisol

Cortisol is one of the essential hormones involved in bone homeostasis. Cortisol helps in bone resorption. An increase in cortisol for short periods also is sufficient enough for bone mineral density depletion. Hyper-cortisol levels are a direct result of the activated HPA axis. Hypercortisolism has direct effects on bone metabolism and the rate of bone loss. The interleukins that are released locally act upon the plaque and in the process disturb the bone mineral homeostasis. The immune cells such as T cells, macrophages, and other related ones are enriched in gingival connective tissue which results in increased secretion of inflammatory molecules. These inflammatory molecules react with stromal cells, gingival epithelium and periodontium to initiate bone resorption. The osteoclast, the principal bone resorptive cell,
monocyte/macrophage precursors under the
itical cytokines macrophage colony-stimulating
l, and osteoprotegerin. TNF-α, IL-1, an d PGE2 also
t activity, particularly in states of inflammatory
those found in periodontitis. The pathogenic
uctive inflammatory periodontal diseases are
gingival plaque microflora and factors such
rides derived from specific pathogens. Host
mmune cell influences propagate these, and the
B cells initiates the adaptive immune response
e Th1-Th2-Th17 regulatory axis.[10]

JECTIVES

bjectives of the present study were to compare
response (CAR), i.e., the difference between cortisol
he level 30 minutes after waking up in aggressive
ealthy subjects. It also conducted to find out cortisol
ned as the difference in the cortisol level between
up in the morning and 1 hour before sleep in
ntitis patients from healthy subjects.

MATERIALS AND METHODS

The ethical approval was obtained from the ethical committee Ajman University, UAE where the study was carried out. A total of 60 patients were grouped into two groups. The age range of the patients was between 18–65 years. All the patients divided into two groups as follows:

- Group 1 to 30 subjects–normal healthy subjects (control)
- Group 2 to 30 subjects with aggressive periodontitis

Exclusion Criteria

- Patients who have any systemic disease other than the ones investigated for female patients who are pregnant/likely to be pregnant.
- Patients who are having any adverse habits like tobacco smoking-chewing, bruxism, alcohol) which can affect the said study.
- Patients (female) who are on any contraceptives based on steroids.
- Patients who are taking any corticosteroids or its derivatives for any diseases.

Inclusion Criteria

All the patients who were diagnozed with clinical radiographically as aggressive periodontitis.

They were examined from university student clinics mentioned in the application form in the age range of 18–45 years. The salivary cortisol was evaluated with electrochemiluminescence immunoassay method (ECLIA) using Cobas-e-411 autoanalyzer by Roche, USA.

All the patients were clinically examined for the periodontal status using the periodontal probe and subjected to radiological examination for assessing the bone loss, oral hygiene index simplified (OHI-S), attachment loss (CAL)from the cemento-enamel junction.BMI was calculated using the formula—kg/m^2

The questionnaire was given to classifying the patients using the Hamilton anxiety scale (HAM-A). Waist circumference was also measured for each patient. This calculation will yield a comprehensive score in the range of 0–56. A score of 17 or less indicates mild anxiety severity. A score from 18–24 indicates mild to moderate anxiety severity, and a score of 25–30 indicates a moderate to severe anxiety severity.

Whole saliva was collected by a passive drooling method from each subject in sterile vials 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 was 2 mL. All the subjects were motivated to participate in the study by signing the patient participation and consent form. The saliva was collected in 3 timings on the same day to find out the pattern of circadian rhythm, at soon after waking up before brushing, 30 minutes after waking up and then the last sample is taken 1 hour before sleeping. The collected samples were stored in a home refrigerator at 3–5° overnight and handed over to Ajman University the next day morning. The samples were transported to the biotechnology lab to be frozen under –20°C. The sample size was 30 subjects in each group with three different readings (Sample A–soon after waking up, Sample B–30 minutes after waking up, sample C–1 hour before sleeping) and hence would accumulate 180 sa mples to be tested in total. The salivary cortisol was evaluated with ECLIA using Cobas e–411 Autoanalyser by Roche, USA.

Results

Descriptive statistics and analysis of variance (ANOVA) analysis were used in this study, the ANOVA analysis used here to study the variation between the study groups and to test if there are significant differences between these groups.

In this study, the mean age of the patient in control group was 24.633 with SD 2.870 while in aggressive patients was 37.33 with SD of 7.288. The BMI showed a higher mean value of 32.113 with SD of 3.949 in aggressive periodontitis compared to control patients with a mean value of 24.428 with SD of 1.871. Moreover, the waist circumference in aggressive periodontitis patients showed a higher mean value of 91.1 with SD of 21.531 while the mean in the control patients was 76.6 with SD of 10.575, and it can be seen that the mean of anxiety 29.59 with SD of 4.368 for aggressive patients while the mean for the control patients was 19.566 with SD of 3.450.

The control patients were selected without any bone loss or with loss of attachment. Aggressive periodontitis patients were having mean bone loss 4.933 with SD of 1.552 also the loss of attachment (mean 5.333 and SD 1.347). The OHI-S values were showing a higher mean value of 3.576 with SD of 1.202 in aggressive periodontitis compared with control patients (mean—1.329 and SD—0.463) (Table 1).

We found that the BMI statistically different between the study groups. Also, there was a significant difference between the study groups on the saliva A (soon after waking up) at the significant level ($\alpha = 0.05$), while there were no significant differences for saliva B and saliva C when we compared between the control and aggressive patients. Moreover, there were significant differences between the study groups in the Cortisol surge or elevation after 30 minutes. Finally, the average cortisol was also found to be statistically different between the groups (Table 2).

From Graph 1, it can be seen that there are significant differences between the two groups for the saliva A (soon after waking up). Also, there was no significant difference (at alpha = 0.05) between control and aggressive patients with regard to saliva B even though the descriptive statistics showed an opposite trend in Aggressive patients. We also observed a lot of out-layered values in saliva b in aggressive patients.

Moreover, there was no significant difference between the groups with regard to saliva C, but again there are many extreme values for the aggressive patients. From Graph 2, we can see that the cortisol elevation was found to be significantly different between the two groups with more value seen in aggressive periodontitis patients. As from Graph 3, the average cortisol in Aggressive patients was found to be higher compared to control patients and significantly different with a *p* value of 0.012.

The cortisol gap and cortisol surge after waking up showed the opposite trend in aggressive patients compared to control patients (Table 3 and Graph 4). When statistically analyzed, cortisol gap did not show any statistical difference between the groups. The reason may be due to the wide variation in values displayed in aggressive patients. But the descriptive statistics displayed a negative trend for the cortisol gap and cortisol surge in aggressive periodontitis. Another interesting finding in aggressive periodontitis patients, there are many values which are falling away from the mean and out layered compared to the values seen in control patients.

Control group is moderately skewed to the left (-ve skewness graph) which means that the left tail is longer and most of the distribution is at the right side. By contrast, the second group (aggressive periodontitis) is moderately skewed to the right (+ve skewness graph) that is; its right tail is longer and most of the distribution is at the left side. Higher kurtosis is observed in

Table 1: Descriptive statistics of the clinical parameters

		Age	*BMI*	*Waist circumference*	*HAM-A*	*Bone loss*	*CAL*	*OHI-S*
Control	Mean	24.633	24.428	76.6	19.566			1.329
	SD	2.870	1.871	10.575	3.450			0.463
AP	Mean	37.333	32.113	91.1	29.590	4.933	5.333	3.576
	SD	7.288	3.949	21.531	4.368	1.552	1.347	1.202

BMI, Body mass index; HAM-A, Hamilton's anxiety scale; CAL, Clinical attachment loss; OHI-S, Oral hygiene index -simplified

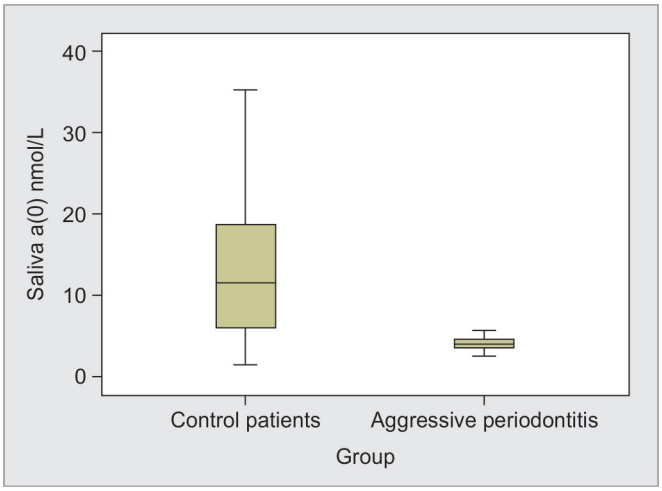
Aggressive periodontitis because of more variance and extreme deviations in the values obtained (Graph 5 and Table 4). The diurnal variation seen in cortisol for the control patient compared to the Aggressive patients. It was observed that the saliva A in control patients started at a low level compared with aggressive patients. Another finding is that the majority of patients showed a negative slope instead of showing a positive slope within 40 minutes after waking up. In normal healthy individuals, the expected cortisol awakening response was shown, but in the aggressive periodontitis cases, there was a negative slope followed by surge causing saliva C values at a higher value 1 hour before sleep. This makes aggressive patients with a higher average of cortisol in saliva compared to control subjects. Since the values for saliva C is higher in aggressive patients the difference between the saliva C–saliva A values (cortisol gap) showed a negative trend in aggressive patients compared with control patients (Table 5 and Graph 5).

Discussion

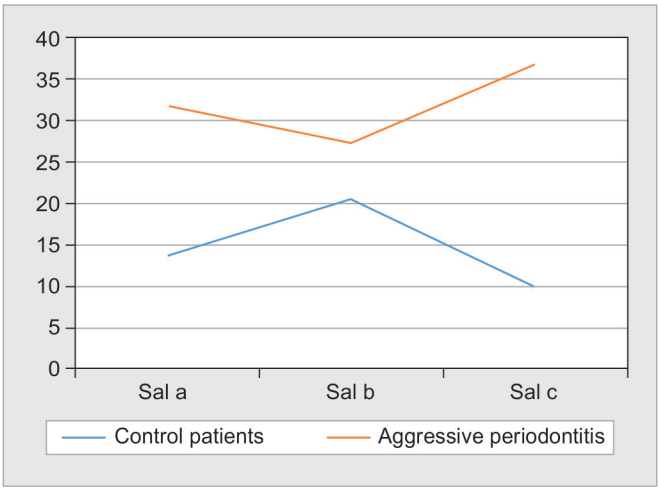
The present study is done to see the effect of the release of cortisol from the adrenal cortex on periodontal tissues. After the data

Table. 2: ANOVA analysis of different parameters

			Sum of squares	*Df*	*Mean square*	*F*	*Sig.*
BMI * group	Between groups	(Combined)	885.965	1	885.965	92.762	0
	Within groups		553.957	58	9.551	–	–
	Total		1439.922	59	–	–	–
Saliva A(0) nmol/l * group	Between groups	(Combined)	1398.189	1	1398.189	27.359	0
	Within groups		2964.064	58	51.105	–	–
	Total		4362.252	59	–	–	–
Saliva B(30) * group	Between groups	(Combined)	1854.482	1	1854.482	1.745	0.192
	Within groups		61640.25	58	1062.763	–	–
	Total		63494.732	59	–	–	–
Cortisol elevation * group	Between groups	(Combined)	6385.398	1	6385.398	19.515	0
	Within groups		18977.394	58	327.196	–	–
	Total		25362.792	59	–	–	–
Saliva C(1HR BS) * group	Between groups	(Combined)	2900.792	1	2900.792	2.213	0.142
	Within groups		76012.572	58	1310.562	–	–
	Total		78913.363	59	–	–	–
Average Cortisol * group	Between groups	(Combined)	7279.74	1	7279.74	6.758	0.012
	Within groups		62480.659	58	1077.253		
	Total		69760.399	59			
Cortisol gap * group	Between groups	(Combined)	1243.424	1	1243.424	0.582	0.449
	Within groups		123933.44	58	2136.784	–	–
	Total		125176.87	59	–	–	–



Graph 1: Comparison of saliva A in nmol/lit (soon after waking up).



Graph 2: Diurnal variation of salivary cortisol

being interpreted, it has shown a clear opposite distinct trend in these pathological diseases. The cortisol awakening response or cortisol surge after 30 minutes waking up has shown in aggressive periodontitis a dip instead of a surge in the present study. The other observation we found that the saliva, 1 hour before sleep has also shown a relatively high value instead of showing a gradual decline during the day time. The limitation of the study was the sample size and the study period.

The salivas A and C were found to be statistically significant when it was compared between the groups. Other statistically

Table 3: Comparison of cortisol gap versus cortisol surge

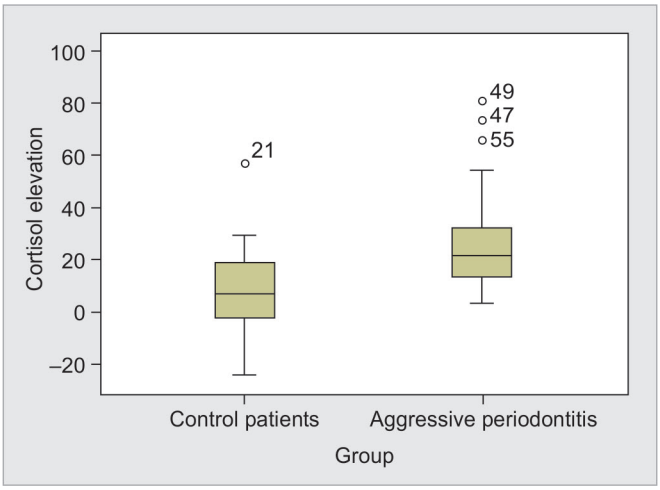
	Cortisol gap	*Ccortisol surge*
Control	3.981667	6.691
Aggressive periodo ntitis	–5.123	–4.187333333

Table 4: Descriptive statistics for the cortisol gap by groups

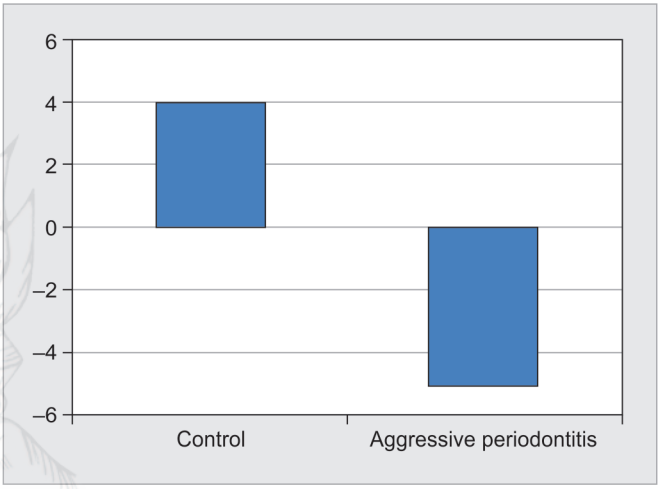
	Group		*Statistic*
Cortisol gap	Control patients	Mean	3.98167
	95% confidence Interval for Mean	Lower Bound	–0.84101
		Upper Bound	8.80434
	5% trimmed mean		4.46370
	Median		4.60500
	Variance		166.807
	Std. deviation		12.915368
	Minimum		–30.460
	Maximum		25.800
	Range		56.260
	Interquartile range		19.307
	Skewness (to the left)		–0.489
	Kurtosis		0.318
	Aggressive periodontitis	Mean	–5.12300
	95% confidence interval for Mean	Lower bound	–29.05236
		Upper bound	18.80636
	5% trimmed mean		-5.74500
	Median		2.73000
	Variance		4106.760
	Std. deviation		64.084010
	Minimum		–176.940
	Maximum		197.490
	Range		374.430
	Interquartile range		41.520
	Skewness (to right)		.229
	Kurtosis		3.926

Table 5: Diurnal variation of salivary cortisol between the groups

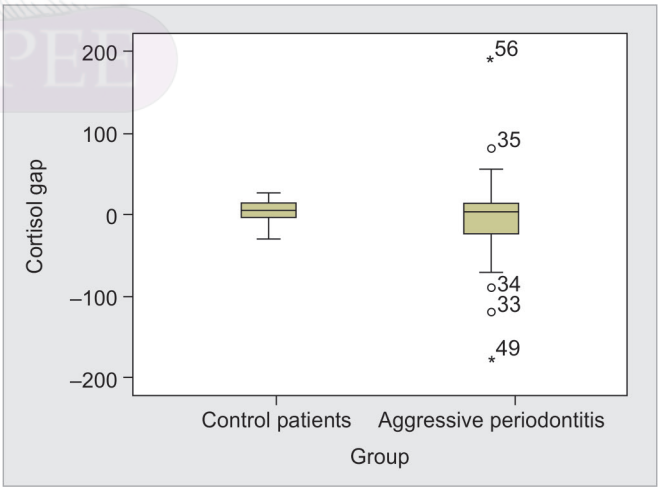
	Sal A	*Sal B*	*Sal C*
Control	13.700	20.391	9.719
Aggressive periodontitis	31.510	27.323	36.633



Graph 3: Comparison of cortisol elevation (CAR) between the groups



Graph 4: Negative trends of cortisol gap in aggressive periodontitis



Graph 5: Comparison of cortisol gap and out layered values in aggressive periodontitis

ligand (RANKL) synthesis by preosteoblast /stromal cells, supporting osteoclast differentiation and net bone resorption.[27]

This study can be extended to include more sample size with the inclusion of more parameters in future to get more accurate results to prove that altered HPA axis could be included as an etiological factor which modifies the host response of an individual.

Conclusion

The following conclusions are drawn from the study. Saliva cortisol is a very accurate tool to measure the HPA axis dysregulation in periodontal pathology. It has been observed that the mean salivary cortisol value is higher in patients with aggressive periodontitis. The cortisol awakening response seen in control patients is not observed in aggressive periodontitis. Instead of giving a surge, the cortisol showed a dip in the first 30 minutes followed by a gradual increase in aggressive periodontitis instead of decline as observed in normal patients. The saliva C sample (collected 1 hour before sleep) also showed a higher steady value in aggressive periodontitis patients compared with control patients. The cortisol gap which is calculated as the difference saliva A value (soon after waking up) minus saliva C value (1 hour before sleeping) has shown a negative trend in the aggressive periodontitis patients.

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