

## Original Research Article

# Estimation of serum cortisol levels and its correlation with salivary cortisol levels in coronary artery disease patients with and without periodontitis: a cross sectional study

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## ABSTRACT

**Background:** Atherosclerosis refers to combination of changes in the intima of arteries, consisting of focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue, calcium deposits and associated medical changes. Ischemic heart disease (IHD) refers to a group of closely related syndromes that is caused by an imbalance between myocardial oxygen demand and blood supply.

**Methods:** A cross-sectional study was conducted on 63 patients, aged 30-65 years, with known coronary artery disease. Estimation of serum and salivary cortisol levels were assessed with specific ELISA kit for cortisol (R&D Systems) and their comparison was performed in patients with and without periodontitis. Statistical analysis was done using Mann-Whitney U test and Pearson Correlation test.

**Results:** The clinical parameters showed statistically significant association ( $p < 0.001$ ) between coronary artery disease and periodontitis and comparative levels of serum and salivary cortisol displayed varying degrees of positive correlation.

**Conclusions:** Saliva along with serum has great potential as a diagnostic fluid and it showed good correlation with cortisol levels in coronary artery disease patients with and without periodontitis.

**Keywords:** Coronary artery disease, HPA axis dysfunction, Periodontitis, Salivary cortisol, Serum cortisol

## INTRODUCTION

After two decades of research, it has been firmly established that an association exists between cardiovascular disease (CVD) and periodontal disease.<sup>1</sup> Marchand introduced the term "atherosclerosis" describing the association of fatty degeneration and vessel stiffening.<sup>2</sup> Both experimental and clinical studies have provided evidence that the disease is driven by an antigenic response to modified lipoproteins, other self-molecules or even microorganisms.<sup>3</sup> In addition, non-

antigenic dependent factors, like metabolic and neuroendocrine functions, may contribute to the inflammatory process.<sup>4</sup>

Chronic stress has been linked to the development of insulin resistance and deposition of abdominal fat, risk factors for coronary artery disease (CAD) and diabetes in both humans and other primates.<sup>5</sup> Acute cardiovascular events and paradoxical arterial vasoconstriction are frequently triggered by physical or mental stress in susceptible patients.<sup>6</sup> Dysregulation of the hypothalamic-

pituitary–adrenal (HPA) axis is one of the pathways through which chronic stress may affect coronary heart disease (CHD) risk.<sup>7</sup> The HPA axis is involved in the containment of immune-mediated inflammatory reactions. It is activated by pro-inflammatory mediators to secrete corticotropin-releasing hormone in hypothalamus, followed by adrenocorticotropin hormone secretion from the pituitary and cortisol secretion by the adrenal glands.<sup>4</sup> Cortisol measurements directly capture the status of HPA axis functioning which is a mediator of many secondary outcomes, ultimately leading to disease.<sup>8</sup> In healthy individuals, cortisol has a distinct diurnal pattern with the peak cortisol occurring in the early morning, declining throughout the day, and reaching a nadir around two or three am.<sup>7</sup> The physiological effect of cortisol is immunomodulatory rather than solely immunosuppressive, causing a shift of cytokine production from a primarily pro-inflammatory to an anti-inflammatory pattern. A dysfunctional HPA axis function may thus involve a failure to contain inflammatory activity in CAD patients, thus providing link between stress and inflammation in disease.<sup>4</sup>

Periodontitis is a bacteria driven chronic inflammatory disease affecting the supporting structures of the teeth. Additionally, microorganisms involved in periodontal diseases can play a role in the formation of atheromatous plaques and given that periodontal disease is one the most common infectious diseases, it can be considered as an important risk factor for CVD.<sup>9</sup>

Over the past two decades, the use of saliva, rather than blood or urine, to determine various adrenal hormone values has gained increasing acceptance and is the method of choice for current stress research. The available literature clearly suggests that salivary cortisol is more closely correlated with the free cortisol fraction in serum compared to total serum cortisol.<sup>10</sup>

While the primary understanding of the HPA axis can be gained by taking three or four salivary cortisol measurements throughout the day, the morning measurement may be the most informative and critical since it provides the largest value to the cortisol levels.<sup>11</sup> Hence, this cross sectional study was undertaken to estimate the levels of serum cortisol and its correlation with salivary cortisol in CAD with and without periodontitis.

## METHODS

This study was conducted for a period of ten months at the outpatient Department of Cardiology, A.J. Institute of Medical Sciences and outpatient Department of Periodontology, A. J. Institute of Dental Sciences, Mangalore, Karnataka in accordance with the Helsinki Declaration of 1975, as revised in 2000. The protocol for the study was approved by the Institutional Ethics Committee, and informed consent was obtained from all the participating subjects. A total of 63 (24 females and

39 males) subjects with known coronary artery disease (CAD), aged between 30-65 years, satisfying the inclusion criteria were selected. Informed consent was taken from all the participants. They were divided into two groups. Test group, had 30 subjects with history of CAD and 30% periodontal pockets with the probing pocket depth of  $\geq 5$ mm in each quadrant of the oral cavity, and control group, had 33 patients with history of CAD without periodontitis.

A proforma was used to record demographic data of the patient regarding age, sex, address, oral hygiene habits and medical history of cardiovascular disease, angina, myocardial infarction, blood pressure recording, ECG and fasting lipid profile. Periodontal status assessment was done by recording plaque index (PI) (Silness and Loe, 1964), gingival index (GI) (Loe and Silness, 1963), clinical attachment level (CAL) and probing pocket depth (PPD) for each individual patient. Smokers and individuals who consume alcohol and subjects with positive history of diabetes mellitus, cerebrovascular, or kidney disease or any known conditions for which prophylactic antibiotic treatment was required before dental examination were excluded.

Ten ml of blood from the cephalic vein and five ml of unstimulated saliva were collected from all 63 hospitalized patients, who were diagnosed with acute coronary syndrome (MI/unstable angina), on the fourth day of admission between eight am and ten am. After centrifugation, the collected serum and saliva was stored at  $-70^{\circ}\text{C}$ . Both serum and saliva samples were analyzed using the Sensitive Enzyme-Linked Immunosorbant Assay (ELISA) kit for cortisol (R&D Systems) at the Department of Microbiology, NGS Maratha Mandal Institute of Dental Sciences & Research Centre, Belgaum.

The statistical analysis was performed using SPSS Version 20 and statistical significance was defined as p value  $<0.05$ . The demographic analysis for distribution of age and sex was performed using students independent t-test and chi-square test respectively. The comparison of test group and control group with each of the clinical parameters, probing pocket depth (PPD), clinical attachment loss (CAL), plaque index (PI) and gingival index (GI) was performed using the Mann-Whitney U test, which was preferred over the students independent t-test due to extreme distribution of values in the data obtained. The correlation between the serum cortisol levels and salivary cortisol level, with respect to each other was performed using Pearsons Correlation test.

## RESULTS

There was no statistical significance observed between the two groups with regard to age distribution (p-value – 0.49) and sex distribution (p value – 0.064) as seen in (Table 1).

The comparison between both groups with the clinical parameters, performed using Mann-Whitney U test, it was observed that the median value in each of the four clinical parameters showed a statistically significant

increase in test group (CAD with Periodontitis), when compared to control group (CAD without Periodontitis) as seen in (Table 2).

**Table 1: Clinical characteristics of study subjects.**

Variables	Control group (CAD without Periodontitis)	Test group (CAD with Periodontitis)	p-value
	n = 33	n = 30	
Age	53.52±8.047	52.2±6.865	0.49
Sex distribution (M/F)	24/9	15/15	0.064

**Table 2: Comparison of the effect of gingivitis and periodontitis on clinical parameters and its significance using Mann-Whitney U test.**

	Control group					Test group					Mann-Whitney U	Z	P-value
	Min	P 25	Median	P 75	Max	Min	P 25	Median	P 75	Max			
Serum cortisol (ng/ml)	0.18	11.5	17.14	33.18	412	32.42	150	287	326	454	64	-5.932	<0.001
Salivary cortisol (ng/ml)	0.16	0.88	1.23	1.86	10.5	0.55	2.25	9.25	24.5	46.5	141.5	-4.865	<0.001
Probing pocket depth	0	0	0	3	4	0	4	4	5	8	82.5	-5.862	<0.001
Clinical attachment loss	2	3	3	4	6	3	4	4	5	7	165	-4.749	<0.001
Gingival index	0.2	0.5	0.7	1.2	1.8	0.6	1.3	1.55	1.8	2.7	159.5	-4.627	<0.001
Plaque index	0.2	0.9	1.4	1.7	2.7	0.7	1.5	1.8	2.3	3	234.5	-3.593	<0.001

Min – Minimum; P 25 – 25<sup>th</sup> Percentile; P 75 – 75<sup>th</sup> Percentile; Max – Maximum.

An increased median is suggestive of increase in the severity of the particular parameter. This signifies that in periodontitis, there occurs an increase in the depth of the pockets, increased loss of attachment of the involved teeth, increased severity in plaque formation and increased inflammation of the gingiva, when compared to control group.

The correlation of both serum and salivary cortisol, assessed by Pearsons Correlation test, showed statistically significant correlation between the level of cortisol and the increasing severity of the clinical parameter. There is good correlation between the increase in probing pocket depth (PPD) and both salivary and serum cortisol, while there is very good correlation between increased loss of clinical attachment and serum and salivary cortisol. Both gingival index and plaque index show good correlation with serum cortisol levels, but shows better correlation with salivary cortisol levels, as observed in (Table 3). This is reinforced when we

observe the scatter graphs comparing serum and salivary cortisol levels, with each of the clinical parameters. The scatter graphs depict lines going upward, which means that with increase in the severity of the clinical parameters, there occurs a rise in the serum cortisol (Figure 1) and salivary cortisol (Figure 2) levels, thus showing a significant increase in serum and salivary cortisol levels in relation to the test group, when compared to the control group.

The correlation between the serum cortisol levels and salivary cortisol levels, with respect to each other, performed using Pearsons Correlation test showed very good correlation, which was statistically significant. It was inferred that there occurs corresponding rise of salivary cortisol levels in response to rise in serum cortisol level (Table 4). This is confirmed from a scatter graph obtained from the study values, where a line going upwards is obtained, which is indicative of a positive correlation (Figure 3).

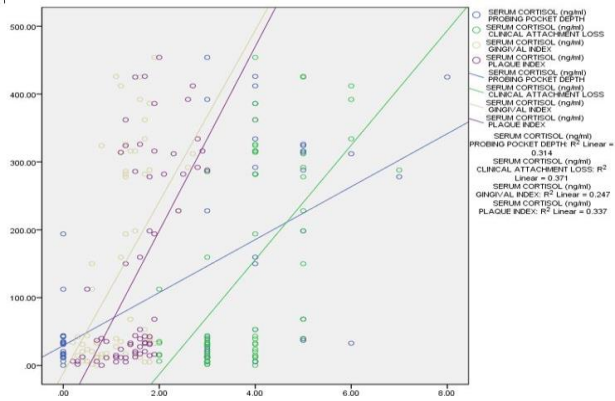


Figure 1: Scatter graph depicting positive correlation between serum cortisol and clinical parameters.

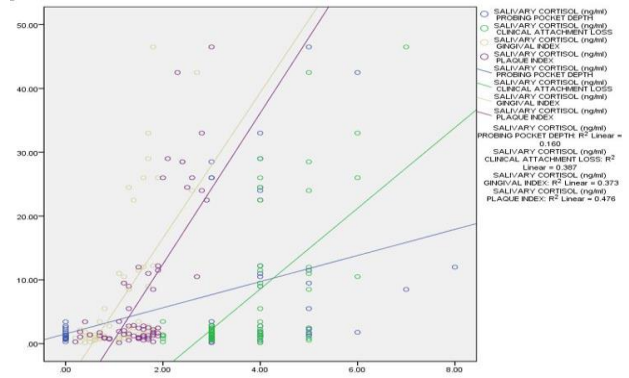


Figure 2: Scatter graph depicting positive correlation between salivary cortisol and clinical parameters.

Table 3: Comparison of correlation between serum and salivary cortisol with clinical parameters.

		Serum cortisol (ng/ml)	Salivary cortisol (ng/ml)
Probing pocket depth	Pearson correlation	0.560	0.400
	Sig. (2-tailed)	<0.001	0.001
Clinical attachment loss	Pearson Correlation	0.609	0.622
	Sig. (2-tailed)	<0.001	<0.001
Gingival index	Pearson Correlation	0.497	0.611
	Sig. (2-tailed)	<0.001	<0.001
Plaque index	Pearson Correlation	0.581	0.690
	Sig. (2-tailed)	<0.001	<0.001

**Pearsons correlation:** Poor correlation 0-0.2; Fair correlation 0.2-0.4; Good correlation 0.4-0.6; Very good correlation 0.6-0.8; Excellent correlation 0.8-1.0.

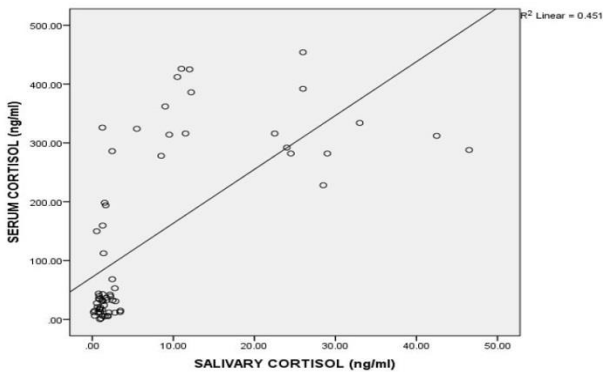


Figure 3: Scatter graph depicting positive correlation between salivary and serum cortisol.

Table 4: Comparison of correlation between serum and salivary cortisol.

		Salivary cortisol (ng/ml)
Serum cortisol (ng/ml)	Pearson Correlation	0.672
	Sig. (2-tailed)	<0.001

Pearsons correlation: poor correlation 0-0.2; fair correlation 0.2-0.4; good correlation 0.4-0.6; very good correlation 0.6-0.8; excellent correlation 0.8-1.0

## DISCUSSION

Atherosclerotic cardiovascular disease (ACVD) is a complex multifactorial disease and may present with one or more combination of risk factors. Periodontitis has been considered to be an independent risk factor, which may increase the risk of future ACVD events.<sup>12</sup> Periodontitis measured using clinical attachment loss, probing pocket depth and radiographic assessment of bone loss has been associated with increased risk for various measures of ACVD along with established cardiovascular risk factors.<sup>13</sup>

There is a definite circadian activity of levels of cortisol with maximum secretion in the morning which declines by evening. Patients with both stable and unstable conditions of CAD exhibited an altered cortisol pattern compared with healthy controls, involving hypercortisolism and a flat diurnal slope due to high evening cortisol values and higher total 24-hour cortisol output.<sup>14,15</sup> In addition, when exposed to acute physical or psychological acute stress, the patients showed a blunted cortisol response.<sup>14</sup> Positive association between cortisol and cholesterol levels have been demonstrated in individuals with CAD in multiple studies.<sup>16-18</sup>



In this study, serum samples were collected between eight am and ten am and the normal range was taken as 16.8-75.5 ng/ml as per the ELISA kit. On evaluation, the values ranged from 0.18 ng/ml to 454 ng/ml, with a mean value of 138.47 ng/ml. It was observed that the level of serum cortisol mirrored the severity of inflammation of the gingiva. This is reflected in the observation that only two cases in control group had a serum cortisol value above the upper limit of normal, while 24 of the 30 cases of test group had higher than normal values, which is statistically significant ( $p < 0.001$ ).

Comparisons between serum cortisol and the levels of cortisol in saliva and urine has been under evaluation for a considerable amount of time, in a quest to find a noninvasive, less expensive and definite alternative. Measurement of biomarkers in saliva offers many advantages, as the procedure is stress-free and non-invasive, and allows for frequent and rapid sampling. A number of studies have quantified that cortisol in saliva reflects serum levels with good precision and is advocating salivary cortisol as an even better alternative to assess dynamic HPA axis activity.<sup>15,19-22</sup> Early morning samples of free cortisol in saliva obtained with strict reference to the time of awakening and measured repeatedly, have shown good intra-individual stability.<sup>23</sup> The salivary cortisol levels, in this study, has reflected serum cortisol levels in most patients, showing very good correlation similar to other studies.<sup>19,20,23</sup> This implies that salivary cortisol can be an alternative diagnostic tool to assess severity of periodontitis, especially in patients with coronary artery disease.

When comparing the clinical parameters in both groups, it was observed that all the parameters were statistically significant in the test group compared to the control group. This result is similar to studies by Mannem et al, Domingues JEG et al and Mudrika et al.<sup>24-26</sup> There was a positive association between the clinical parameters and the levels of both serum and salivary cortisol. The association showed statistically significant correlation ranging between good to very good between the level of cortisol and with the increasing severity of the clinical parameter. There is good correlation between the increase in probing pocket depth (PPD) and both salivary and serum cortisol, while there is very good correlation between increased loss of clinical attachment and serum and salivary cortisol. Both gingival index and plaque index show good correlation with serum cortisol levels, but shows better correlation with salivary cortisol levels, when both are compared. This result is similar to those obtained by Nayak SU et al, Mudrika S et al, Genco RJ and Hilgert JB.<sup>26-29</sup> Clinical attachment levels can be regarded as a result of an inflammatory burden from the past into the present, in contrast to probing pocket depth level which reflects the current pathophysiological status of periodontitis and thus, the findings from our study may be attributed to dysregulation of the HPA axis, which is chronically activated in patients with periodontitis.

## CONCLUSION

It was concluded that cortisol levels, both serum and salivary, can be used to assess the severity of periodontitis and that serum cortisol and salivary cortisol levels have good correlation with one another. Thus, salivary cortisol can be considered as a non-invasive and cost effective alternative test to serum cortisol to assess severity of periodontitis in patients with coronary artery disease.

The study may have been underpowered, due to the small sample size. The severity of CAD and the impact of its treatment may have reflected on the cortisol levels but it was not quantified or differentiated from the cortisol rise due to periodontitis – both in serum and in saliva. Multiple samples taken in the same patient over consecutive days and averaged for assessment of cortisol levels, could have provided a more accurate result and an evening sample could have provided an insight into the diurnal variation of serum and salivary cortisol, both of which was not performed in this study.

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