VOLUME-6 | ISSUE-2 | FEBRUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

EVALUATION OF ASSOCIATION BETWEEN PSYCHOLOGICAL STRESS AND CORTISOL LEVELS IN PATIENTS WITH CHRONIC PERIODONTITIS.

Dental Science

KEYWORDS: Periodontitis, Serum and salivary cortisol, Psychological stress.

DR. ROSHNI JAISWAL — III Year post graduate student, Department of Periodontics, AB Shetty Memorial Institute of Dental Sciences, Mangalore. NITTE University

DR. NINA SHENOY — PROFESSOR, Department of Periodontics, AB Shetty Memorial Institute of Dental Sciences, Mangalore. NITTE University

ABSTRACT

Background and objectives: Periodontal diseases are the diseases affecting the supporting structures of teeth. While the primary agent is bacteria, within the subgingival biofilm, causing periodontal tissue destruction, the progression of the same is modified by a variety of environmental factors like stress eliciting an inappropriate host response.

Psychological stress is implicated as one of the significant risk factor for periodontal disease. Excessive stress leads to activation of HPA axis leading to immuno-suppression and also brings about behavioural changes favouring at-risk health behaviours. These two mechanisms may have long term deleterious effect on periodontal tissues. Cortisol, a steroid hormone produced by the adrenal gland is a well-established stress biomarker. Serum and salivary cortisol values are bound to increase in stressful conditions; hence it is a reliable indicator of psychological stress. Hence this study was designed to evaluate and co-relate the levels of serum and salivary cortisol and oral hygiene status in stressed subjects with chronic periodontitis.

Method: The present study was conducted in the Department of Periodontics, A.B. Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore. The study comprised of 90 stressed subjects suffering from chronic periodontitis. Oral hygiene Simplified and periodontal parameters like probing pocket depth and clinical attachment levels were recorded. Saliva and blood samples were collected and were analyzed to estimate the cortisol levels. Results obtained were then statistically analyzed to estimate mean and standard deviation and co-relation was evaluated using Pearson's co-relation test.

Results: The results of the study demonstrated that stressed subjects had higher mean values of serum and salivary cortisol with poor oral hygiene status. Moreover, statistically significant co-relation was found between periodontal parameters i.e. probing depth and clinical attachment levels with the increased cortisol values ($p<0.05$) indicating that psychological stress may induce periodontal tissue destruction.

Conclusion: Results of this study highlight that stressed individuals present with poor oral hygiene status, which may lead to periodontal destruction. Furthermore, a significant association was found between cortisol values and periodontal parameters indicating stress as a probable risk factor which might influence the severity of periodontal disease. Though clinical trials with larger sample size are required to establish a definite conclusion.

INTRODUCTION

Stress is defined as the psycho physiological response of the organism to a perceived challenge. It originates from a Latin word "stringere" which means "tight," or "strained". In 1976, Seyle elaborated stress further, as the response state of an organism to physical and mental forces beyond the adaptive capacity that lead to diseases of adaptation and eventually to exhaustion and death. He recognized stressors which act to produce positive changes in the body (e.g. exciting, pleasurable), leading to a response state which he defined as "eustress", or stressors could be negative that induce, threatening homeostasis with pain, discomfort and physical pathology. He defined the negative response state as" distress".[1]

Stress is compatible with good health, being necessary to cope with the challenges of everyday life. Problems start when the stress response is inappropriate to the intensity of the challenge and overpowers the host.[2]

Various reasons of stress can be drastic life changes, financial worries, competition at work, health related issues and personal relationships.

Stress has its bearing on health as it can down regulate the cellular immune response in three different ways[3]:

1) Hypothalamo-pituitary-adrenal (HPA) axis
2) Peripheral release of neuropeptides
3) Sympathetic nervous system (SNS) via the release of adrenaline and nor adrenaline.

Hypothalamus-pituitary-adrenal cortex axis is directly affected during stressful condition leading to its prolonged activation which can be deleterious to health and may provide a link between physical illness and mental stress.[4]

Certain behavioural responses to stress can also amplify the risk of disease.Individuals under stress are more likely to engage in behaviours with compelling ramifications for health, including altered sleeping and eating habits and ample consumption of alcohol and other substances .[5]

Moreover, various clinical observations and epidemiologic studies have found that host defence and vulnerability to oral inflammatory infectious diseases are influenced by psychological factors and tension of behavioural and emotional challenge induced by life events.[6,7].

Various stress related oral problems have been listed which includes, dental caries/erosion, gingivitis,periodontitits, bruxism, myofacial pain dysfunction syndrome, apthous ulcers, oral lichen planus and burning mouth syndrome etc.[8]

The potential relationship between stress and oral inflammatory infectious diseases is not a new concept; psychological stress was known as a predisposing factor in the aetiology of necrotising ulcerative gingivitis for more than four decades.[9]

The contribution of psychological factors to the development and progression of periodontal disease has recently become an area of increased research activity. Periodontitis is an inflammatory response of the periodontium, which involves obliteration of investing tissues surrounding the teeth consequently leading to loss of tooth support and eventually tooth loss. Although, pathogenic bacterias are the foremost reason to initiate the course of disease, it is now apparent that their habitation alone is not sufficient determinant of periodontal destruction.[10-12]

Host response to plaque accumulation is modified in the presence of multitudinal systemic or environmental factors such as, stress, depression and anxiety due to which periodontal disease process may become more destructive.[13-17]

The role of stress in periodontal disease has a persuasive

pathophysiological base.

Of the proposed mechanisms, that strives to accomplice psychosocial factors to periodontitis, the negligence of oral health care behaviour was thought to be crucial. This hypothesis is predicated on the postulation that stressed patients due to reduced motivation and concern, disdain from oral hygiene maintenance and professional routine dental treatment. [18]

Futhermore, stress is also associated with pernicious habits like smoking and alcohol dependence. These two factors are known to intensify one's risk for chronic periodontitis. [19, 20] Another probable mechanism, whereby stress may be an additional risk factor to the development and progression of disease, is through variations in host immune response, making the individual more liable to develop long-term unhealthy status impacting periodontal health.[21]

Stress can be measured by various approaches. Psychologists have developed questionnaires that cover an extensive range of psychological symptoms that can be induced by exposure to chronic stress and brings about a transition in personality, cognition and emotions. It can also be assessed by physiological means i.e.by evaluating stress hormones like cortisol and catecholamines in blood, urine and saliva. [22]

Cortisol, a steroid hormone produced by the adrenal gland is a well-established stress biomarker. The normal range for serum cortisol is 4-20 μg/dL in a morning specimen. [23] It is the preeminent hormone in stress and fight or flight response secreted from the pituitary gland and regulated by adrenocorticotropic hormone. Cortisol values can be affected by physical stress, emotional stress and illness. In case of high level of stress, serum cortisol value is bound to increase, thus it is an unequivocal indicator in patients experiencing stressful situations in life. [24]

Saliva is considered as an imperative component of the host oral immune defense system. It can emulate changes in oral and systemic health; hence it is an effective source for estimating various biomarkers. Many immune response biomarkers have been found in saliva, and some of these were demonstrated to be involved in periodontal disease, such as matrixmetalloproteinases-8 and -9, osteoprotegerin, and interleukin-1b, choromogranin A, cortisol, and α-amylase.Salivary Cortisol levels reflect the activation of HPA. It has many advantages such as; the collection procedure is non-invasive, stress-free and allows for rapid sampling. It has become the most prominent biomarker illustrating psychological stress with a reference range of 5-10 ng/mLin normal condition. [25]

Even though investigators have reviewed the impact of psychological components on the extent and severity of periodontitis and its subsequent effect on the immune system, very few studies exist that demonstrate the impact of stress and cortisol on the oral hygiene status and health of the periodontium.

Hence, an attempt was made in present study to evaluate the association between psychological stress and cortisol levels in patients with chronic periodontitis.

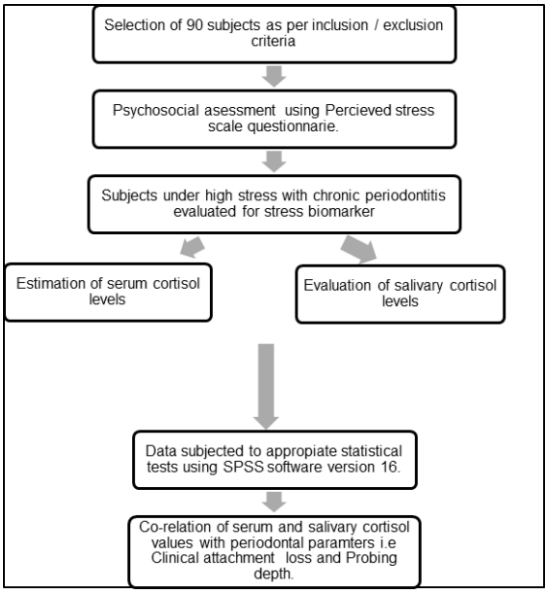
Aim

The aim of this study was to evaluate and co-relate the levels of serum and salivary cortisol and oral hygiene status in stressed subjects with chronic periodontitis.

The objectives of this study were:

1. To assess cortisol levels in serum and saliva of individuals with chronic periodontitis.
2. To evaluate oral hygiene status of the stressed subjects with chronic periodontitis.
3. To evaluate the association between cortisol levels in stressed subjects with chronic periodontitis.

Approach



METHODOLOGY

Source of the data:

90 subjects were recruited from the routine OPD at the Department of Periodontics, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore, Karnataka.

Informed was obtained from all the participants after explaining the nature and purpose of the study.

Sample size was calculated using the following formula:

$$N = \frac{2Sp^2[Z1-\alpha/2 + Z1-\beta/2]^2}{\mu d^2}$$

α- level of significance
Sp^2- pooled variance
1-β- power
μd- clinical significant difference

METHOD OF COLLECTION OF DATA

Study sample

The study included 90 stressed subjects diagnosed with chronic periodontitis.

Screening examination

1. Participants demographic details such as age, income, family history of periodontal diseaseand frequency of brushing were recorded. Subjects were asked to mention if they avoid oral health care during stress.

2. Perceived Stress Scale; by Cohen et al [26] was used for the evaluation of stress by the subjects on 5 point scale basis.

CRITERIA FOR SELECTION

Inclusion criteria:

1. Male and female subjects within the age group of 25-50 years with a minimum complement of 20 teeth.

2. Subjects diagnosed with chronic periodontitis as per International Workshop for Classification of Periodontal Diseases and Conditions [27] i.e. the involvement of (>30% sites) with moderate to severe clinical attachment loss (>3mm), measured with William's periodontal probe.

3. Subjects who scored 20 points or higher on a Perceived stress scale, indicating high stress.

VOLUME-6 | ISSUE-2 | FEBRUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

Exclusion criteria:
1. Patients on corticosteroid therapy , antibiotics , immuno-suppressive drugs, tranquilizers, sedatives and anti-depressants,who underwent periodontal treatment six months before examination. with salivary gland dysfunction. pregnant or lactating women and subjects who smoke and consume alcohol.

PERIODONTAL PARAMETERS

1. Mean probing pocket depth: The pocket depth was measured by using Williams's graduated periodontal probe at six sites i.e. distobuccal, midbuccal, mesiobuccal, distolingual, midlingual and mesiolingual of each tooth. All six measurements were added and divided by the number of sites examined i.e. 6 to obtain the mean probing depth for an individual tooth.

2. Mean clinical attachment level: Clinical attachment level was measured on six surfaces per tooth i.e. distobuccal, midbuccal, mesiobuccal, distolingual, midlingual and mesiolingual.

3. Oral Hygiene Index: Simplified (OHI-S) [28] was recorded for each subject.

METHOD OF SAMPLECOLLECTION

SALIVA COLLECTION

Saliva was collected by passive drool technique between 9am-11am as during this time cortisol is within detectable range and gives stable values. It was allowed to pool at the bottom of the mouth and transferred into the collection device directly. Analysis of results can be done easily as this technique gives a trouble free sample of high quality.

To avoid bacterial growth it was transferred to containers and stored at -20° C.The samples were refrigerated within 30 minutes within 4 hours after collection. After collection from subjects samples were transported in the same containers for analysis of cortisol levels within 24 hours of collection.

COLLECTION OF BLOOD SAMPLE: About 3-5 ml of blood was collected from the subjects between 9-11am from the median cubital vein by venipuncture.

ESTIMATION OF CORTISOL IN SALIVA

Estimation of Cortisol level in saliva was done by using Diametra ELISA KIT by direct immune enzymatic technique.

REFERENCE VALUES

The following values can be used as guideline.

A.M.	3 – 10 ng/mL
P.M.	0.6 – 2.5 ng/mL

ESTIMATION OF COTISOL IN SERUM: Blood was centrifuged and then serum cortisol level was evaluated using Cortiso ELISA Kit by fully automated bidirectionally interfaced chemiluminescent immune assay technique.

STATISTICAL ANALYSIS

SPSS(Statistical package for social sciences) version 16.0 was used for Statistical analysis. Values were represented asmean ±standard deviation.

To study the correlation between serum and salivary cortisol levels with periodontal variables Pearson's test was performed and Pearson's correlation coefficient was obtained). p value of <0.05 was considered statistically significant.

ARMAMENTARIUM

- Mouth mirror
- Tweezers
- Williams periodontal probe
- Cotton swabs
- Mouth mask
- Disposable latex gloves
- Kidney tray
- No.23 explorer(Shepherd's hook)
- Plain and EDTA test tubes
- ELISA Kit

PHOTO I: ARMAMENTARIUM FOR CLINICAL EXAMINATION.



PHOTO II : CLINICAL PICTURE OF PROBING WITH WILLIAM'S PERIODONTAL PROBE



PHOTO III: COLLECTION OF VENOUS BLOOD



PHOTO IV: Diametra Salivary cortisol ELISA kit



PHOTO V: Serum cortisol ELISA kit



RESULTS

This cross sectional study included 90 stressed subjects with chronic periodontitis. Descriptive statistics i.e. mean and standard deviation of the parameters studied was calculated and illustrated with suitable tables. (Table 1-4)

Pearson's co-relation co-efficient was determined .Positive value of "r" represents positive co-relation between 2 variables which is graphically represented with the help of scatter diagrams. (Graph 1-4) .Results were considered significant at ($p < 0.05$) Calculation of oral hygiene index-simplified scores revealed that the participants had poor oral hygiene status, with the mean value of 4.79±0.93. [Table1 (S.no.1)].

Mean values for periodontal parameters i.e. probing depth and clinical attachment loss was computed to be 7.30±1.11 mm and 4.07±1.01 mm respectively, as demonstrated in [Table 1 (S.no.2&3)]. According to perceived stress scale, mean stress score was calculated to be 22.1±1.19 which distinctly affirm that subjects were under high stress as show in [Table 1 (S.no. 4)].

As represented in [Table 2(Sno.1)] the mean serum cortisol level was 23.68±1.91 µg/dL which is higher than the reference range i.e. 6.2-19.4 µg/dL. (7-10 AM)

Similarly the mean salivary cortisol level as depicted in [Table 2 (S no.2)] was estimated to be 13.46±1.98 ng/mL, which is well above the reference range as mentioned in the kit manual i.e. 3-10 ng/mL.

In a co-relation analysis between serum cortisol and periodontal parameters, positive values of Pearson's co-relation co-efficient "r" i.e. 0.077 and 0.108 for probing depth and clinical attachment loss respectively, highlights a positive association between both the variables. In addition, this co-relation is statistically significant. (p values 0.047 and 0.038 respectively). [Table 3]

Correspondingly, on interpreting the co-relation between salivary cortisol levels and periodontal parameters, estimated using Pearson's co-relation test, positive values of Pearson's co-relation co-efficient "r" i.e. 0.45 and 0.53 for probing depth and clinical attachment level respectively, revealed a positive association between both the variables. Furthermore, this co-relation is highly significant statistically for both periodontal parameters (p value of < 0.001*). [Table 4]

Table 1: Mean values of Oral Hygiene index, Periodontal Parameters and Perceived stress scale scores

S.No.	Parameters	Mean	Standard Deviation
1.	OHI-S	4.79	0.93
2.	Probing depth (mm)	7.30	1.11
3.	Clinical attachment loss (mm)	4.07	1.01
4.	Perceived stress scale scores	22.1	1.19

Table 2: Mean values of Cortisol levels in serum and saliva of patients

S.No	Variables	Mean	Standard Deviation
1.	Serum cortisol levels(µg/dL)	23.68	1.91
2.	Salivary cortisol levels(ng/mL)	13.46	1.98

Table 3: Co-relation b/w Serum cortisol levels and periodontal parameters

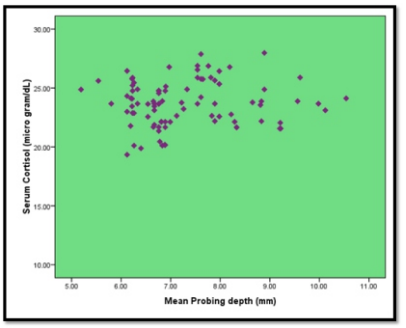
S.No.	Variables	Pearson's co-relation	p value
1.	Serum cortisol level & PD	0.077	0.047*
2.	Serum cortisol level & CAL	0.108	0.038*
(*indicates significant at 5% level of significance)			

PD=probing depth,CAL = clinical attachment loss

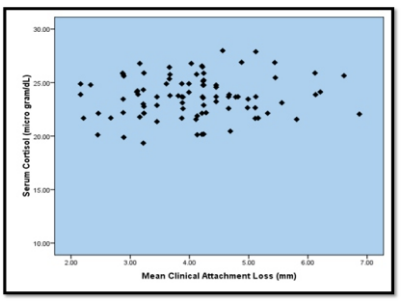
Table 4: Co-relation b/w salivary cortisol levels and periodontal parameters

S.No.	Variables	Pearson's co-relation,r	p value
1.	Salivary cortisol level & PD	0.45	< 0.001*
2.	Salivary cortisol level & CAL	0.53	< 0.001*
(*indicates significant at 5% level of significance)			

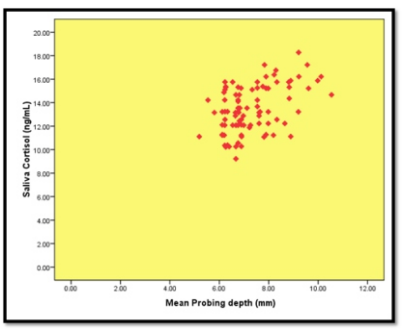
PD=probing depth, CAL = clinical attachment loss



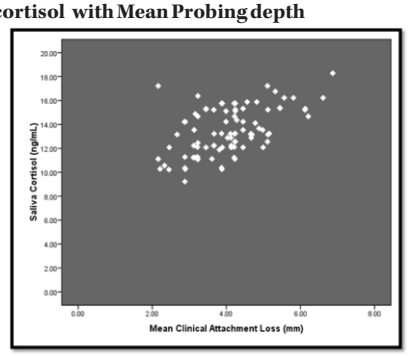
Graph I: Scatter diagram depicting co-relation between serum cortisol values with Mean Probing depth



Graph II: Scatter diagram depicting co-relation between serum cortisol values with Mean Clinical Attachment loss



Graph III: Scatter diagram depicting co-relation between salivary cortisol with Mean Probing depth



Graph IV: Scatter diagram depicting co-relation between salivary cortisol with Mean Clinical Attachment loss.

DISCUSSION

Chronic stress alters the potency of the immune response by having a net negative effect, resulting in lopsidedness between host-parasite

VOLUME-6 | ISSUE-2 | FEBRUARY-2017 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 78.46

interaction where parasites take over, ultimately leading to periodontal breakdown. [29]

This study sheds light on the probable association between stress and periodontal parameters by assessing cortisol levels in both serum and saliva of patients.

The mean oral hygiene score reveals poor oral hygiene status of the subjects who participated in the study [Table 1 (S.no.1)]. This is in congruence with the behavioural model highlighting the role of stress in periodontal disease proposed by Genco, stating that stressful situations bring about behavioural modifications, which perturb at-risk health behaviours such as poor oral hygiene, smoking and poor compliance with dental care .Long term avoidance of oral hygiene leads to plaque build up which is detrimental to periodontal tissue. There is also a possibility that stress triggers overeating, especially a high-fat diet, which elevates cortisol production leading to immune-suppression, hence further worsening the periodontal health. [5]

Co-incident findings were reported by Deinzer et al, who examined the role of academic stress in medical students during exams on periodontal health. It was concluded that stress-induced neglect of oral hygiene might be partly responsible for periodontal destruction thus adding evidence to the assumption that stress may adversely affect oral hygiene performance and quality.[30]Various other studies have reported ignorance of oral hygiene during stressful period. [31-33]

The high values of mean probing depth and mean clinical attachment loss indicate increased severity of periodontal disease. [Table 1 (S.no. 2&3)].The mean perceived stress scale scores distinctly enlighten the fact that all subjects were under immense stress. [Table 1 (S.no.4)]. The cortisol levels in saliva and serum were also well above the reference range justifying the fact ,that participants were subjected to high stress. [Table 2 (S.no.1&2)]. These findings can be elucidated by the biologic model implying that increased level of steroids results in decreased resistance to infection by annihilating IgA, inducing immuno-suppression due to which early colonization of periodontal pathogens is favoured ,hence leading to intensification of disease. [4] Moreover , in a stressful state there is an escalation in cortisol production which decrease the number of circulating lymphocytes, monocytes and eosinophils impeding the accumulation of macrophages and neutrophils at inflammatory sites suppressing the cascade of the immune response.[34] Hence, these variations have considerable subduing actions on the inflammatory responses leading to marked susceptibility to periodontal infection. Co-relative evidence was presented in a systematic review conducted by Peruzzo et al. where, out of 14 studies, the majority (57%) reported a positive relationship between stress and periodontal disease. This review demonstrated that the majority of the work published investigating this inter-relationship has veritably found compelling evidence. [35]

On determining the association between cortisol levels and periodontal parameters, this investigation revealed statistically significant association between the two. (Table 3, Graph 1&2)(Table 4, Graph 3 &4).Persistent chronic inflamed state leads to adrenal activation and secretion of high levels cortisol causing profound immunosuppression. [36] Therefore, with increasing chronicity of a stressor ,the adaptiveness of immune system changes appears to decrease .[37]This influence the course, onset, and outcome of infectious pathologies tilting the balance towards more aggressive form of disease hence justifying increased periodontal breakdown in the form of clinical attachment loss and probing depth.[38] Also, prolonged inflammatory state favours a TH2 dominant response, which is responsible for progressive periodontal disease.[39] Similar findings were presented in studies conducted by , Genco et al [4]and Ishisaka et al.[40] who reported higher mean cortisol levels in patients with the periodontal disease and stress.

Conversely,Mengel et al.did not report significant correlation between cortisol and stress. [41]

Hypercotisolemia can induce the production of pro-inflammatory cytokines secretion such as interleukin-1 2, 3, 6 and tumor necrosis factor alpha (TNF-α). [42]. Similar findings were reported, showing an association between increased salivary cortisol and periodontal parameters.[43-45]

The results of this cross sectional study showed that, stressed subjects present with poor oral hygiene status, which can ultimately lead to destruction of periodontium. Moreover it can be concluded that there is a noteworthy association between increased serum and salivary cortisol values and the severity of periodontitis, validating that excessive stress may jeopardize the periodontal health.

Hence stress can be identified as one of the strategic factor accountable for destructive periodontal disease and should be addressed for the welfare of the patients.

CONCLUSION

- The present study depicts that participants under stress had high cortisol values and poor oral hygiene, which may have adverse effects on periodontium. Additionally, significant association was found between stress and severity of periodontal disease.

- However, certain limitations such as , the cross-sectional nature of the study did not reveal the true effect of stress over the course of periodontal disease. In addition, healthy group was not taken into consideration.

- Hence there is, still a scope to conduct future studies with larger samples and repeated measurements of variables following a longitudinal approach for irrefutable evidence.

REFERENCES

1. Selye, H. (1976) Stress in Health and Disease. Butterworths, Boston
2. Goyal S, Gupta G, Thomas B, Bhat KM, Bhat GS. Stress and periodontal disease: The link and logic!!. Ind Psychiatry J 2013; 22:4-11.
3. Mahendra L, Austin RD, Kumar SS, Mahendra J, Felix AJ. Relationship between psychological stress, serum cortisol, expression of MMP-1 and chronic periodontitis in male police personnel. Int J Sci Eng Res 2012; 3(9):2-5.
4. Genco RJ, Ho AW, Kopman J, Grossi SG, Dunford RG, Tedesco LA. Models to evaluate the role of stress in periodontal disease. Ann Periodontol1998; 3(1): 288-302
5. Conway TL, Vickers RR, Ward HW, Rahe RH. Occupational stress and variation in cigarette, coffee and alcohol consumption. J Health Soc Behav.1981; 22(2):156–65.
6. Croucher R, Marcenes WS, Torres MC, Hughes F, Sheiham A. The relationship between life-events and periodontitis. A case-control study. J Clin Periodontol 1997; 24: 39-43.
7. Breivik T, Thrane PS, Murison R, Gjermo P. Emotional stress effects on immunity, gingivitis and periodontitis. Eur J Oral Sci 1996; 104: 327-334.
8. Bhushan K ; Prabhdeep K Sandhu ,Shaiba Sandhu. Psychological stress related oral health problems- dental perspective. IJRID 2014; 4 (3):43-47.
9. Johnson BD, Engel D. Acute necrotizing ulcerative gingivitis. A review of diagnosis, etiology and treatment. J Periodontol 1986; 57: 141-150.
10. Williams RC. Periodontal disease.N Engl J Med 1990; 322(6):373-82.
11. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. J Periodontal Res 1993; 28(6 Pt 2):500-10.
12. Johannsen A, Asberg M, Söder PO, Söder B. Anxiety, gingival inflammation and periodontal disease in non-smokers and smokers-an epidemiological study. J ClinPeriodontol 2005; 32(5):488-91.
13. Haber J, Kent RL. Cigarette smoking in a periodontal practice. J Periodontol 1992; 63(2):100-6.
14. Beck JD. Methods of assessing risk for periodontitis and developing multifactorial models. J Periodontol 1994; 65(5 Suppl):468-78.
15. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE,Norderyd OM, Genco RJ,et al. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J Periodontol 1994; 65(3):260-7.
16. Offenbacher S. Periodontal diseases: Pathogenesis. Ann Periodontol 1996; 1(1):821-78.
17. Vettore MV, Leão AT, Monteiro Da Silva AM, Quintanilha RS, Lamarca GA. The relationship of stress and anxiety with chronic periodontitis. J ClinPeriodontol 2003; 30(5):394-402.
18. Kurer JR, Watts TL, Weinman J, Gower DB. Psychological mood of regular dental attenders in relation to oral hygiene behaviour and gingival health. J ClinPeriodontol1995; 22(1):52-5.
19. Breslau N, Kilbey MM, Andreski P. Nicotine dependence and major depression. New evidence from a prospective investigation. Arch Gen Psychiatry 1993; 50:31-5.
20. Marmorstein NR. Longitudinal associations between alcohol problems and depressive symptoms: Early adolescence through early adulthood. Alcohol ClinExp Res. 2009; 33(1):49-59.
21. Irwin M, Patterson T, Smith TL, Caldwell C, Brown SA, Gillin JC, Grant I.et al. Reduction of immune function in life stress and depression. Biol Psychiatry 1990; 27(1):22-30.
22. Ice, G.H. and James, G.D. (eds.) Measuring Stress in Humans: A Practical Guide for the Field.2006.Cambridge University Press.
23. Chernecky CC, Berger BJ. Cortisol - plasma or serum.Chernecky CC, Berger BJ, eds. Laboratory Tests and Diagnostic Procedures. 6th ed. Philadelphia, PA: Elsevier Saunders; 2013:388-89.

24. Rai B, Kaur J, Anand S, Jacob R. Salivary Stress Markers, Stress, and Periodontitis: a pilot study.JPeriodontol 2011; 82(2):287-92.
25. Haririan H, Bertl K, Laky M, Rausch WD, Böttcher M, Matejka M, Andrukhov O, Rausch-Fan X, et al. Salivary and Serum Chromogranin A and a-Amylase in Periodontal Health and Disease. J Periodontol. 2012; 83(10):1314-21.
26. Cohen S,Kessler R.C. & Underwood Gordon,L. Strategies for measuring stress in studies of psychiatric and physical disorders. A guide for health and social scientists.NewYork, Oxford University Press; 1995.3-26.
27. 1999 International Workshop for a Classification of Periodontal Diseases and Conditions. Papers. Oak Brook, Illinois, October 30- November 2, 1999. Ann Periodontol 1999; 4(1): i, 1-112.
28. Greene JC, Vermillion JR. The Simplified Oral Hygiene Index. J Am Dent Assoc.1964; 68:7–13.
29. Green LW, Tryon WM, Marks B, Juryn J. Periodontal disease as a function of life-events stress. J Human Stress 1986; 12(1): 32–6.
30. Deinzer R, Ruttermen S, Mobes O, Herforth A. Increase in gingival inflammation under academic stress. J ClinPeriodontol 1998; 25(5):431-3.
31. Deinzer R, Foster P, Fuck L, Herforth A, Stiller- Winkler R, Idel H et al. Increase of crevicular interleukin 1b under stress at experimental gingivitis sites and at sites of perfect oral hygiene. J ClinPeriodontol 1999; 26(1): 1-8.
32. Deinzer R, Kottman W, Foster P, Herforth A, Stiller-Winkler R, Idel H. After effects of stress on crevicular interleukin-b. JClinPeriodontol 2000; 27(1): 74-7.
33. Deinzer R, Hilpert D, Bach K, Schawacht M, Herforth A. Effects of academic stress on oral hygiene--a potential link between stress and plaque-associated disease? J ClinPeriodontol. 2001 ;28(5):459-64.
34. Glassman A, Miller G. Where there is depression, there is inflammation . . . sometimes! Biol Psychiatry2007 ;62(4):280-1.
35. Peruzzo DC, Benatti BB, Ambrosano GM, Nogueira-Filho GR, Sallum EA, Casati MZ, Nociti FH Jr, et al. A systematic review of stress and psychological factors as possible risk factors for periodontal disease. J Periodontol 2007; 78(8):1491-1504.
36. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. Ann N Y Acad Sci.2012;1261: 55–63.
37. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull 2004; 130(4): 601–30.
38. Biondi M, Zannino LG. Psychological stress, neuroimmunomodulation, and susceptibility to infectious diseases in animals and man: a review. Psychother Psychosom 1997; 66(1): 3–26.
39. Breivik T, Opstad PK, Gjermo P, Thrane PS. Effects of hypothalamic- pituitary- adrenal axis reactivity on periodontal tissue destruction in rats. Eur J Oral Sci. 2000; 108(2):115-22.
40. Ishisaka A, Ansai T, Soh I, Inenaga K, Awano S, Yoshida A, Hamasaki T, Sonoki K, Takata Y, Nishihara T, Takehara T et al. Association of cortisol and dehydroepi and rosterone sulphate levels in serum with periodontal status in older Japanese adults. J ClinPeriodontol 2008; 35(10):853-61.
41. Mengel R, Bacher M, Flores-De-Jacoby L. Interactions between stress, interleukin-1beta, interleukin-6 and cortisol in periodontally diseased patients. J ClinPeriodontol 2002; 29(11):1012-22.
42. Salvi GE, Lawrence HP, Offenbacher S, Beck JD Influence of risk factors on the pathogenesis of periodontitis. Periodontol 2000.1997; 14: 173-201.
43. Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA Relationship of stress, distress, and inadequate coping behaviors to periodontal disease. J Periodontol 1999; 70(7): 711-23.
44. Mannem S, Chava VK. The effect of stress on periodontitis: a clinicobiochemical study. J Indian SocPeriodontol 2012; 16(3): 365–9.
45. Abhishek N Zingade.Stress, Depression and Cortisol. Is our Periodontium vulnerable? Int J Adv Res 2014: 2(4):575-80.