Research Article

Evaluating the clinico-biochemical association between stress and chronic periodontitis by estimation of serum cortisol and serum chromogranin-A levels

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Aim. Periodontitis is a persistent, long-standing condition of the tissues holding the teeth which manifests itself as loss of attachment. Numerous systemic, environmental as well as psychological factors have the capability to deteriorate the host's immune response leading to more severe periodontal destruction. Alterations in immune, neural, endocrinal and behavioral systems caused by stressful life events may impair periodontal health. This study was designed to investigate the link between periodontal infection and psychoneuroimmunologic variables by using serum stress markers.

Materials and Method. 400 systemically healthy patients in the age group of 20-60 years were employed for this study. These subjects were segregated into 2 groups namely the healthy periodontium group (200 subjects) and the chronic periodontitis group (200 subjects) after recording plaque index, probing depth, gingival index and clinical attachment loss. The analysis of stress levels by using the Social readjustment rating scale and lifestyle evaluation by using the Health Practice Index was done. Serum cortisol and serum chromogranin-A levels were recorded. Descriptive statistics and an Unpaired t-test for comparing the differences in the parameters amongst groups were done. Pearson's correlation test was performed for establishing a correlation between serum markers with clinical variables and stress levels. MANCOVA analysis to find the linear relation of periodontal and serum parameters (dependent variables) with psychological stress and lifestyle patterns (independent variables) was done.

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Results. Poorer lifestyle and higher magnitude of stress were more allied to worsened periodontal condition ($p \leq 0.05$) than with healthy periodontium. Significantly higher ($p \leq 0.05$) concentrations of serum cortisol, as well as serum chromogranin-A levels, were observed in the chronic periodontitis group thus suggesting stress to be a risk profile in subjects with chronic periodontitis.

Conclusion. Stress shall be regarded as a probable risk factor for periodontal infection and henceforth, it should be catered to so as to attain benefits in patients with advanced and inexplicable periodontal disease.

Introduction

Periodontitis is a chronic infectious disease damaging the supporting structures around teeth which is presumed to be primarily caused by a bunch of microorganisms leading to the destruction of alveolar bone and periodontal ligament and resulting in a gingival recession or pocket formation or both[1]. Its onset and progression are affected by systemic status, local factors, environmental conditions and behavioral patterns that may modify the host resistance to infecting periodontal micro-organisms[2]. Imbalances in all these variables lead to interruption in tissue homeostasis resulting in repetitive cycles of damage and repair. Numerous epidemiologic studies conducted worldwide have reported that mostly 5% to 20% of any community or population present with severe forms of periodontitis while usually the moderate form is presented in maximum percentage in the adult population. An oral health survey conducted on the Indian population revealed that the overall prevalence of periodontal disease in adults is 51% (CI: 41.9-60.1) whereas gingivitis is prevalent in 46.6% of the population. Every second individual who is more than 35 years is suffering from the moderate form of periodontitis and about 35% of extractions were done owing to underlying periodontal diseases[3].

Since periodontitis is a destructive disease with inflammation being the most prominent feature like other common chronic diseases, its progression is also affected by numerous modifiable as well as non-modifiable risk factors through a variety of biological and behavioral mechanisms. Over the years, the role of the bio-psychosocial model for disease progression is being considered over the conventional and most acceptable theory of biological processes being the only cause of physical illness. This hypothesis considers that systemic illnesses take place due to complex interactions between biological, socio-cultural and psychological factors[4]. Stress in any form whether physiological, pathological, traumatic or psychosocial has always been found to be significantly

related to inflammatory diseases with underlying impaired wound healing. It also says that stress may act as either a predisposing factor or a precipitating factor or else a perpetuating factor in the causation of oral diseases[5].

Stress has been defined as a "process in which environmental demands strain an organism's adaptive capacity resulting in both psychological as well as biological changes that could place any individual at risk for illness". The amenability to this condition can be affected by many underlying elements such as genetic traits, personality type, social support and coping styles. It is experienced when an imbalance occurs between environmental events and a person's coping ability[6].

The contribution of stress in the progression of periodontitis is assumed to be because of numerous reasons. Under physiological conditions, adaptation via autonomic, endocrinal, metabolic and immune responses happens but with chronic stress dysregulation of these pathways takes place leading to significant biological damage[7]. Chronic stress (CS) is the reaction of the hypothalamic-pituitary-adrenal (HPA) axis to the extended time frame of physiological as well as psychological activities taking place in or around an individual. These stress-induced responses can result in behavioral changes which can trigger the secretion of corticotropin-releasing hormone (CRH) from the hypothalamus followed by activation of the pituitary gland through the hypothalamic-pituitary-adrenal (HPA) axis[8]. This triggering of the master gland causes an increase in adrenocorticotropic hormone (ACTH), causing the release of corticosteroids from the cortex of adrenal glands with the simultaneous activity of the adrenomedullary system (SM) resulting in the secretion of catecholamines[9].

These actions of stress may cumulate and contribute to the progression and eventual deterioration of periodontal disease as the upsurge of cortisol reduces the number of circulating neutrophils, diminished chemotaxis and phagocytosis along with a lowered number of pro-inflammatory cells. The decrease in the differentiation of cells of adaptive immunity like killer, helper and cytotoxic lymphocytes with decreased proliferation of IgG and IgA antibodies forming B cells and natural killer cells has been observed[6]. Reduced secretory IgA and IgG antibodies, impaired neutrophil function, modulation of Toll-like receptors, with elevated inflammatory cytokines like IL-1 and matrix metallo-proteinases production causes rapid periodontal tissue destruction. Altered T-helper and T-killer cell ratio and increased values of Tumor Necrosis Factor (TNF-α), cytokines, prostaglandins and other proinflammatory molecules[8] are observed.

Apart from HPA axis activation, sensory nerve fibers activation results in the release of neuropeptides occur which act as neurogenic promoters, thereby causing the release of cytokines. Chromogranin-A (Cg A), one of the members of the family of acidic proteins stored in the adrenal medulla is co-released with adrenaline and nor-adrenaline. It is one of the neuropeptides which is very stable and is said to be highly associated with mental stress[10].

With increased immunosuppression, there is an upsurge in the number of dysbiotic bacteria. The increased levels of cortisol lead to an increase in the number of Porphyromonas gingivalis causing dysbiosis of the periodontal microbiome which magnifies the destruction caused by periodontitis[11].

The influence that stress puts on underlying tissue varies according to type, frequency and duration of stress as well as on the potential of a particular personality to cope with it. Subjects who are stressed, tend to adopt negative behavioral and lifestyle changes like neglect of oral hygiene, increased frequency and altered smoking patterns with reduced gingival circulation and salivary flow, clenching or grinding of teeth and a few other oral health depreciating habits which may lead to exacerbation of the disease[12].

While conceptualizing stress in contemporary times as an intricate part of disease etiology, stress is measured at different levels of stressors, moderating factors and stress reactions. The effect of stressors or threats is highly dependent on the appraisal process which causes personalized perceptions for that agent. Personality traits are the major mediating factors which can affect physiological responses like hormonal, neurochemical and autonomic changes thus making it an everchanging, reciprocal response of complicated systems with conceptualization, synthesis, implementation or characterization of different integral constituents at various levels.

Hence, considering stress to be an imperative risk factor for inflammatory diseases, this study was conducted to assess the association between chronic periodontitis and stress with an estimation of serum cortisol and serum chromogranin-A levels.

Material and Methods

Selection of Subjects

This comparative, clinical-biochemical, single-blinded, case-control study was conducted on 400 systemically healthy subjects in the age group of 20-60 years irrespective of gender. The study was

approved by the ethics review committee of the institute.

Inclusion Criteria:

1. Age between 20-60 years
2. Systemically healthy subjects

Exclusion Criteria:

1. Patients on corticosteroid therapy
2. Patients on immunosuppressive drugs
3. Patients who were under antibiotic treatment and had undergone periodontal treatment 6 months before the examination
4. Pregnant or lactating patients.
5. Patients who were on tranquilizers, sedatives or antidepressants
6. Subjects not ready to fill out a consent form.

Study Design:

Complete clinical and periodontal examination of the subjects included was carried out. **Plaque index (PI) (Turesky Gilmore Modification of Quigley Hein Plaque Index)**[13] and **Gingival index (GI) (Loe and Silness)**[14] were recorded. Periodontal status was assessed by measuring probing pocket depth and clinical attachment level. Panoramic radiographs were taken to confirm the diagnosis.

The selected subjects were divided into two groups:

Group I (n=200) – Consisted of individuals with healthy periodontium having all teeth with probing depth ≤ 3mm and no bone loss on radiographs.

Group II (n=200) – Consisted of individuals with chronic periodontitis. Periodontitis was defined as patients with probing depth ≥ 5mm or interdental CAL ≥ 1-2 mm in more than 30% of present dentition with radiographic evidence of bone loss.

The oral examination of all patients was followed by the administration of a questionnaire based on the **Social Readjustment Rating scale as described by the Holmes Rahe Stress scale Inventory**[15] **and their lifestyle evaluation was done by the Health Practice Index** as suggested by **Breslow**[16]**.**

Patients were then referred, for **Serum Cortisol** (Chemiluminescent immunoassay) **and Serum Chromogranin-A** (Enzyme-Linked Immunosorbent assay) level assessment. Venipuncture was done to collect 10 ml of blood. The procedure was done only between 09.00 am to 11.30 am and a CLIA test was carried out of the collected blood sample for serum cortisol assessment and ELISA was done for assessment of serum chromogranin-A levels.

The statistical analysis was done using SPSS version 23 statistical analysis software. Descriptive analysis was carried out, as well as the Unpaired 't' test for intergroup comparison of means of all variables. Pearson's Correlation Coefficient for investigating the relationship between quantitative variables was calculated. Multivariate analysis of Covariance (MANCOVA) was carried out to find out the relation between multiple dependent variables with one independent variable.

Results

The present study was a comparative, case-control, single-blind study carried out on 400 systemically healthy patients.

The present study showed the following results:

Groups	Gender		Age in Years	
	Males	Females	Mean	SD
Healthy Periodontium (I)	119	81	31.85	8.67663
Chronic Periodontitis (II)	125	75	44.92	9.54985

Table I. Demographic Comparison between Group I & II

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Groups	Number	Plaque Index		Significant
		Mean	SD	
Healthy Periodontium	200	0.8490	.11342	Significant (< 0.05)
Chronic Periodontitis	200	2.1838	.73982	
Groups	**Number**	**Gingival Index**		**Significant**
		Mean	**SD**	
Healthy Periodontium	200	.4465	.30123	Significant (< 0.05)
Chronic Periodontitis	200	2.4710	.38090	
Groups	**Number**	**Probing Depth**		**Significant**
		Mean	**SD**	
Healthy Periodontium	200	1.6250	.59679	Significant (< 0.05)
Chronic Periodontitis	200	5.7765	.25162	
Groups	**Number**	**Clinical Attachment Level**		**Significant**
		Mean	**SD**	
Healthy Periodontium	200	.4715	.11707	Significant (< 0.05)
Chronic Periodontitis	200	3.4215	.15429	
Groups	**Number**	**SRRS**		**Significant**
		Mean	**SD**	
Healthy Periodontium	200	162.865	16.44381	Significant (< 0.05)
Chronic Periodontitis	200	304.58	12.73635	
Groups	**Number**	**Lifestyle Evaluation**		**Significant**
		Mean	**SD**	
Healthy Periodontium	200	5.4600	.60848	Significant (< 0.05)
Chronic Periodontitis	200	2.3600	.75714	
Groups	**Number**	**Serum Cortisol Level**		**Significant**
		Mean	**SD**	

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Healthy Periodontium	200	7.9543	.88518	Significant (< 0.05)
Chronic Periodontitis	200	25.1400	.95399	

Groups	**Number**	**Serum Chromogranin Levels**		**Significant**
		Mean	**SD**	
Healthy Periodontium	200	61.1630	1.80686	Significant (< 0.05)
Chronic Periodontitis	200	83.2530	.49009	

Table II. Intergroup comparison of Means of all variables of Group I and Group II

Parameters	**Pearson Correlation Value**	**Level of significance (p-value)**	**Pearson Correlation Value**	**Level of significance (p-value)**
	(Group I)		**(Group II)**	
Probing Depth	.025**	(p≥ 0.01) Non-Significant	0.676*	(p≤ 0.05) Significant
Clinical Attachment Loss	.083**	(p≥ 0.05) Non-Significant	0.536**	(p≤ 0.05) Significant
Plaque index	.232**	(p≥ 0.05) Non-Significant	0.762**	(p≤ 0.05) Significant
Gingival index	.166*	(p≥ 0.05) Non-Significant	0.612**	(p≤ 0.01) Significant

Table III. Correlation of Periodontal parameters with SRRS in Group I and Group II

Inference: *Periodontal parameters were positively correlated with stress levels in Group II and collinearity was found to be statistically significant (p≤ 0.05) for all the parameters which indicated a strong positive association between stress and periodontal parameters.*

Parameters	Pearson Correlation Value	Level of significance (p-value)	Pearson Correlation Value	Level of significance (p-value)
	(Group I)		(Group II)	
Probing Depth	-.198*	(p≥ 0.05) Non-Significant	-.648	(p≤ 0.05) Significant
Clinical Attachment Loss	-.012	(p≥ 0.05) Non-Significant	-.534**	(p≤ 0.05) Significant
Plaque index	-.032**	(p≥ 0.05) Significant	-.563**	(p≤ 0.05) Significant
Gingival index	-.165 **	(p≥ 0.05) Non-Significant t	-.781 **	(p≤ 0.01) Significant

Table IV. Correlation of Periodontal parameters with Life Style Evaluation in Group I and Group II

***Inference**: Periodontal parameters were negatively correlated with lifestyle i.e. when lifestyle pattern value decreased periodontal parameters increased. The correlation was significant (p≤0.05) for all parameters but the association was found to be maximum with the gingival index (p≤ 0.01) in Group II.*

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Parameters	Pearson Correlation Value	Level of significance (p-value)	Pearson Correlation Value	Level of significance (p-value)
	(Group I)		(Group II)	
Probing Depth	.054**	(p≥ 0.05) Non-Significant	.247**	(p≤ 0.05) Significant
Clinical Attachment Loss	.012	(p≥ 0.05) Non-Significant	.760**	(p≤ 0.01) Significant
Plaque index	.106**	(p≥ 0.05) Non-Significant	.583**	(p≤ 0.01) Significant
Gingival index	.072**	(p≥ 0.05) Non-Significant	.350**	(p≤ 0.05) Significant

Table V. Correlation of Periodontal parameters with Serum Cortisol Levels in Group I and Group II

Inference: *Periodontal parameters were positively correlated with serum cortisol i.e. with an increase in severity of the periodontal disease the values of serum cortisol also increased but the correlation was found to be statistically significant (p≤ 0.05) in Group II only.*

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Parameters	Pearson Correlation Value	Level of significance (p-value)	Pearson Correlation Value	Level of significance (p-value)
	(Group I)		(Group II)	
Probing Depth	.175**	(p≥ 0.05) Non-Significant	.432**	(p≤ 0.05) Significant
Clinical Attachment Loss	.125	(p≥ 0.05) Non-Significant	.478**	(p≤ 0.05) Significant
Plaque index	.117**	(p≥ 0.05) Non-Significant	.517**	(p≤ 0.05) Significant
Gingival index	.130**	(p≥ 0.05) Non-Significant	.554**	(p≤ 0.05) Significant

Table VI. Correlation of Periodontal parameters with Serum Chromogranin-A in Group I and Group II

Inference: *Periodontal parameters were positively correlated with serum chromogranin-A i.e. with an increase in severity of the periodontal disease the values of serum chromogranin-A also increased and the correlation was found to be statistically significant (p≤ 0.05) in Group II.*

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Independent Variable	Dependent Variable	B	Std. Error	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Stress (Social Readjustment Rating Scale)	Serum Cortisol Levels (mcg/dl)	16.337	1.497	10.913	.001	13.385	19.289
	Serum Chromogranin Levels (ng/ml)	77.768	.737	105.561	.001	76.315	79.221
	Probing Depth	1.717	.421	4.075	.005	.886	2.548
	Clinical Attachment Loss	1.184	.247	4.787	.001	.696	1.671
	Plaque Index	.996	1.116	.892	.005	3.196	1.205
	Gingival Index	.630	.616	1.024	.005	.584	1.844

Table VII. Effect of Stress on various variables of Group I and Group II after adjusting for Age, Gender and Socio-economic status (Multivariate Analysis of Co-variance)

Inference: *Statistically significant positive relation ($p \leq 0.05$) of all periodontal parameters as well as serum cortisol and serum chromogranin-A levels (dependent variables) was found with stress (independent variable). The regression coefficient (B= 77.768) was found to be highest for serum chromogranin-A levels.*

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Parameter Estimates							
Independent Variable	Dependent Variable	B	Std. Error	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Lifestyle Evaluation	Serum Cortisol Levels (mcg/dl)	-24.138	.234	102.954	.000	23.675	24.600
	Probing Depth	-2.735	.065	42.215	.000	2.607	2.862
	Clinical Attachment Loss	-2.296	.039	59.375	.000	2.219	2.372
	Plaque Index	-6.506	.184	35.363	.000	6.143	6.869
	Gingival Index	-2.434	.061	39.685	.000	2.313	2.555
	Serum Chromogranin Levels (ng/ml)	-82.225	.101	814.834	.000	82.026	84.424

Table VIII. Effect of lifestyle pattern on various variables of Group I and Group II after adjusting for Age, Gender and Socioeconomic status

Inference: *Statistically significant negative relation ($p \leq 0.05$) of all periodontal parameters as well as serum cortisol and serum chromogranin-A levels (dependent variables) was found with lifestyle (independent variable). The regression coefficient (B= -82.225) was found to be highest for serum chromogranin-A levels.*

Discussion

Since ages, systemic health and oral health are two integral parts of general health-related quality of life with researchers working avidly for the amelioration of both of them while maintaining a balance between the two. Periodontitis as a condition is very complex and the recognition of associated risk elements for acquiring this disease is continuously under investigation[17]. The development and advancement of this disease are affected by behavioral, systemic, local and social conditions that may modify the immunity and eventually alter the course of the disease. Evidences that point to the

potential of psychosocial stress to downregulate the inflammatory response causing the onset and continuation of such diseases are also mounting.

The understanding of the relative interaction between psychological well-being and general health has increased and so has the significance of the interplay between environmental demands, adaptive capacities, general health and oral health which is now also considered a major part of cumulative health[18]. Theinterplay between periodontitis and mental health has largely been overlooked in oral research. Meanwhile, the necrotizing forms of periodontal disease are found to be related to stress for many years and evidence points out a positive association between the two conditions.

The current interpretation of the stress mechanism revolves around the concept of the general adaptation response must for the survival of any living being. All kinds of stressors whether major or minor are capable of initiating responses to maintain homeostasis[19]. These central or peripheral adaptation reactions have been known to cause physical changes in almost all systems of the human body, including metabolic changes, respiratory and cardiovascular alterations, gluconeogenesis along with lipid peroxidation and diminished growth or behavioral changes which includes the facilitation of pathways that will increase alertness, vigilance and cognition, thus causing containment of the stress response[20].

The principal brain components integrate together to form a synergistic system leading to biochemical changes thereby making the basis for physiological adaptation response: firstly the corticotropin-releasing hormone responds through the hypothalamic-pituitary-adrenal axis followed by locus ceruleus-norepinephrine system causing modulation of the sympathetic adreno-medullary nervous system. These basic neural and systemic networks aim to achieve homeostasis for both the central and peripheral organ systems dysregulated by stress[21][22][23].

The constituent parts of this entire feedback chain collaborate and cooperate with each other, and they do this in an interrelated manner so that the activation of parts of one system can influence the components of another system. Glucocorticoid secretion (from the brain and adrenal cortex) and catecholamines (from thebrain and adrenal medulla) generally follow a circadian rhythm but maintain the homeostasis in presence of stressful situations[24].

The entire immuno- inflammatory apparatus acts as a feedback loop thereby regulating the interaction between the neuroendocrine (hypothalamic-pituitary-adrenal) system with parts of the immune-inflammatory system. A negative feedback loop may increase the activity of

the hypothalamic-pituitary-adrenal system. The entire neuronal system gets stimulated and there is a simultaneous increase in adrenocorticotrophic hormone activity leading to an increase in the production of circulating prostaglandins, cytokines, interleukins, cortisol and other neuroendocrine messenger substances like Chromogranin A (CgA) or DHEA from the adrenal medulla[25][26][27].The presence of neuropeptides is also considered a neurogenic promoter which may further aggravate the production of interleukins and cytokines.

Based on this pathophysiology, a confederation between psychosocial factors and periodontal status is presumed as stress usually affects gingival tissues through decreased gingival circulation, decreased salivary flow rate, altered host resistance and hormonal imbalance[28]. The neuroimmunological effects of stress cause altered cytokine production, varied ratios of T-helper cell phenotypes and TH2 cell dominance which is implicated in causing rapidly destructive forms of periodontitis

Also, it has been seen in experimental studies that with elevated levels of corticosteroids, collagen production, as well as collagen remodelling, is impaired leading to the rapid destruction of tissues, especially in the presence of inflammation. These multiple pathways of interaction between stress and inflammation have led to the assumption of the presence of possible communication pathways between these two conditions[29].

The present study was a randomized, comparative, clinical-biochemical study conducted for analysing the association of stress and chronic periodontitis by means of assessing cortisol and chromogranin- A levels in serum.

The study recruited 400 individuals in two groups in which Group I had Subjects having healthy periodontium and a mean age of 31.85 whereas Group II had subjects with Chronic Periodontitis with a mean age of 44.92 **(Table I).**

Patients aged 20-60 years were chosen as subjects of this age have come across many episodes of untoward life events which can cause an increase in the serum chromogranin-A levels immediately and serum cortisollevels chronically for longer periods thus exerting their deteriorating effects on the periodontium.

Systemically healthy patients were selected for this study as a variety of ailments that are known to alter or may alter the course of periodontitis could have affected the result of the present study in an aberrant manner.

Factors which may negatively influence the results of the study were eliminated while formulating the exclusion criteria for the patients. In the present study subjects taking corticosteroid therapy were excluded because of the structural and pharmacological similarity of these drugs with the endogenous hormone cortisol. Smoking patients were excluded from the study as smoking leads to the turning of the hypothalamo-pitutary-adrenal (HPA) axis which can cause changes with respect to levels of cortisol. A higher nicotine content is found to have a dose-dependent effect on HPA axis hormones, so smoking cigarettes can elevate peak nicotine levels **(Mendelson JH 2008)**[30] thus compounding the situation.

Patients taking antibiotics were excluded as it leads to alteration in serum cortisol levels **(B T Pritchard 2017)**[31]. Pregnant and lactating mothers were also excluded from this study as during pregnancy there is reportedly increased production of steroidal hormones[32]. Also, the placenta starts the production of CRH and ACTH which is autonomous and it is not amenable to normal glucocorticoid feedback control **(Carr BR 1981 et al**[33]**, Petraglia F *et al* 1987**[34]**)**.

Patients who were on tranquilizers, sedatives and antidepressants were not considered for this study as tranquilizers are known to increase HPA axis activity by cyclic AMP-dependent pathway whereas the antidepressants are known to induce an increase in the number of glucocorticoid receptors thus rendering the HPA axis to be more sensitive to glucocorticoid activity[35].

The condition of the periodontal tissues was gauged by a variety of ancillary parameters **(Table II)** namely the **Plaque index (Turesky, Gilmore and Glickman 1970)**[13] **and Gingival index (Loe and Silness)**[14] in both Group I and Group II. The intergroup comparison between Group I **(0.84± 0.11)** and Group II **(2.18± 0.73)** showed a statistically significant **($p \leq 0.05$)** difference in plaque level scores. This difference could be attributed to behavioral changes owing to stress which leads to neglect of oral hygiene.

The intergroup variation in Gingival Index was **($p \leq 0.05$)** significant in Group I **(0.44± 0.30)** and Group II **(2.47± 0.38).** This difference was considered to be due to increased plaque scores in Group II and also a supposed increase in red complex bacteria predominantly Porphyromonas Gingivalis which uses cortisol as its nutrient **(Spector et al 2020)**[35].

The pocket probing depth (PPD) was measured and in the present study Group II **(5.77± 0.25)** was found to have statistically **($p \leq 0.05$)** higher probing depth than **Group I (1.62 ± 0.59) (Table II).**

Clinical attachment level has always been considered a more realistic portrayal of attachment gain or loss than probable pocket depth as it is not influenced by gingival margin levels and in this study, it was observed that **(Table 7 and Graph 7)** clinical attachment loss was significantly higher in Group II (3.42 ± 0.15) **($p \leq 0.05$)** when compared to Group I **(0.47± 0.11)**.

This variation in the parameters of both groups was attributed to the fact that stress causes inhibition of T-cell-mediated immune responses. The increased serum glucocorticoids directly change the antibody-mediated immunity (Th2- mediated response) which elevates the growth of pathogenic periodontal microorganisms. Not only the cellular but also the humoral responses are affected, thus making the subject more amenable to worsened inflammatory conditions like periodontitis.

These results were found to be similar to the studies conducted by **Zoila Refuilo et al (2013)**[36], **Jaiswal R et al (2016)**[37], **Rajhans NS et al (2017)**[38], **Pitzurra L et al (2020)**[39], **Mankar K et al (2021)** [40] **and Petit C et al (2021)**[41].

Stress has been considered an abstract concept that can be measured and quantified with various methods. These methods of quantifying stress may include the use of different stress scales as well as certain biomarkers. For this study, a standardized questionnaire method, **The Holmes- Rahe Stress scale Inventory**(149) (SRRS) measures the cumulative impact of stressful life events that had occurred in the last 12 months. The stress scores on evaluation were considerably statistically higher **($p \leq 0.05$) in Group II (304.24± 12.7)** as compared to **Group I (162.8± 16.4) (Table II)**. The patients in Group II were greater scores according to Holmes Rahe Scale and these results were consistent with those found in studies done by **Dohrenwend B et al (2006)**[42], **and Noone P et al (2017)**[43].

The lifestyle of the patients is considered to have an important modifying role in the pathogenesis of periodontitis as it highlights the personal characteristics of an individual. The **Health Practice Index** was scored to assess the lifestyle as this index represents the overall/ combined aspects of lifestyle related to health. The lifestyle evaluation in this study showed statistically higher values **($p \leq 0.05$)** in Group II **(2.36±0.75)** as compared to Group I **(5.46±0.60) (Table II)**. The poorer lifestyle habits are found to be present in patients with stress which may lead to behavioral changes like neglect of oral hygiene, dietary changes, deleterious oral habits, bruxism, altered gingival circulation, insufficient sleep or alcohol abuse. The results of the study were found to be in agreement with the studies of, **Aleksejuniene (2002)**[44] **and Gundala R (2010)**[45].

Apart from stress rating scales, the quantification of stress was also done by means of assessing biomarkers as they play a critical role in formulating a diagnosis, and prognosis, tracking regression and progression and helping in therapy guidance in stress-related diseases and disorders.

Acute stress biomarkers have been established and validated but when the biomarkers of the chronic stress response longer acting) are evaluated it has been found that when a long-standing stress is present the hypothalamus triggers the anterior pituitary to release adrenocorticotrophic hormone (ACTH) which signals adrenal cortex cells to produce and secrete corticosteroids.

Once the cortisol is secreted it comes out in the bloodstream and exerts its action by binding to cytosolic receptors so the freely available or unbound cortisol due to its inherent lipophilic nature and low molecular weight is found in cells and tissue fluids which can then be easily measured.

In this study, serum cortisol levels were recorded in subjects who reported between 9.00 a.m. to 11.00 am asthis time pattern follows the circadian rhythmicity of cortisol production[46].

Cortisol is the most frequently used marker that is presumed to reflect variations due to chronic mental as well as physical stress, but there are biomarkers which correlate only with mental stress symptoms. **Chromogranin A (CgA)** is one such marker which is novel and is reported to give a better picture of acute stress symptoms. CgA is an acidic glycoprotein reposited in the dense granules of the adrenal medulla and of many other neuroendocrine cells as well as neurons. It belongs to the class of regulated secretory proteins. Also, chromogranins are reportedly much more stable than catecholamines so they may act as a better index of sympatho-medullary activity during the acute stress response.

The present study evaluated serum cortisol and serum chromogranin-A- A levels as stress biomarkers. Assessment of serum cortisol levels was done by the chemiluminescent immunoassay (CLIA) method as it is highly sensitive & has good specificity.

Serum Chromogranin-A levels were assessed by ELISA method (Enzyme-Linked Immunosorbent assay). This method is a more sensitive, rapid and reproducible method without any cross-reactivity with the samples.

In this study, significantly **($p \leq 0.05$)** greater values of cortisol in serum **(25.14 ± 0.95)** were observed in Group II in comparison to Group I **(7.95±0.88) (Table II).** The results were found to be consistent with many previous studies like that of **Satheesh Mannem (2012)[47], Zoila Refuilo (2013)[36], Huaixiu**

Lu (2016)[37], Roshni Jaiswal (2016)[38], Pitzurra L et al (2020)[39], Mankar K et al (2021)[40] and Petit C et al (2021)[41].

The intergroup comparison of serum Chromogranin-A levels in the present study revealed considerably statistically higher **($p \leq 0.05$) levels in Group II (83.25± 0.49) as compared to Group I (61.16± 1.8) (Table II)**. The results were seemingly similar to the results of **Hady Haririan et al (2010)[25], Haririan H et al (2012)[26] et al.**

Statistical analysis was done to find whether the parameters assessed were having any association with each other. Pearson Correlation analysis to find the correlation amongst variables of periodontal health and stress and lifestyle parameters was done. The results revealed that there was a correlation between the Social readjustment rating scale (SRRS) and periodontal parameters in Group I patients whereas the correlation wassignificant ($p \leq 0.05$) between two variables in Group II patients **(Table III).** The studies done by **Rosania AE et al (2009)[27]** also showed similar results.

It was also observed that lifestyle parameters were correlated in Group I patients which was not significant ($p \geq 0.05$) statistically but it was significant ($p \leq 0.05$) in Group II patients **(Table IV).** The results of the study `were found to be parallel with the results of many studies[25].

These results could be attributed to the behavioral model which signifies the effect of psychological stress on lifestyle patterns leading to health-impairing behaviors like neglect of oral hygiene maintenance, anxiety-induced forgetfulness, difficulty in concentrating, disturbed sleeping patterns or increased consumption of cigarettes or alcohol. The irascible behaviour or disturbed sleep patterns result in a reduction of growth hormone which downregulates the tissue repair response.

The correlation analysis revealed that a non-significant ($p \geq 0.05$) relationship was observed between Serum Cortisol and serum Chromogranin-A levels with periodontal parameters in Group I patients while this relationship was found to be statistically significant ($p \leq 0.05$) in Group II **(Table V and VI)** patients.

This association is in sync with the biological model for illnesses which suggests that steroids when increased beyond the physiologic amounts may lead to suppressed immune response leading to impaired host resistance against periodontal pathogens. Additionally, stress leads to overconsumption of diets with higher fat ingredients which further increases the release of cortisol thereby triggering the HPA activation and kick-starting a vicious immuno-inflammatory cascade.

Chromogranin-A secretion is stimulated by stress which simultaneously causes PMN secretion, cell degranulation, oxidase activation and causing the release of a large variety of pro-inflammatory molecules. Also, there is a significant accumulation of nitric oxide and TNF-α with an increase of chromogranin-A thus affecting the progression of periodontal disease. The studies of **Jaiswal R et al (2016)**[38], **Petit C et al (2021)**[41] **Reshma PA (2013)**[48], **Penmetsa G et al (2019)**[49], showed analogous results.

The presence of stress and lifestyle patterns as independent variables affecting periodontal parameters was evaluated by means of multivariable analysis which was conducted using the variance components model **(MANCOVA analysis) (Table VII and VIII).** The periodontal parameters along with serum cortisol and chromogranin-A levels (dependent variables) were found to have a linear relation with psychological stress and lifestyle patterns (independent variables). The covariates of age, gender and socioeconomic status were preadjusted. It was observed that greater scores of psychological stress and poorer lifestyle patterns were analogous with worsened periodontal parameters and serum cortisol and serum chromogranin were also found to increase proportionately.

The maximum association was observed for serum chromogranin-A levels i.e. (β=77.768) and (β= 82.2) with both stress and lifestyle patterns respectively. The results of this study were found to be similar to that of studies done by **Petit C et al (2021)**[41], **and Aleksejuniene J (2002)**[44].

Stress has till now been considered a non-modifiable determinant in the progression of periodontal disease. Variousresearches have been done and an array of studies are still going on to prove the true association between these twohighly prevalent interrelated conditions. The current study also attempted the same with the inclusion of two biomarkers in a considerably higher number of subjects. From the observations obtained from the results, it could be elucidated that stress was most probably affecting the progression of the disease as the other confounding variables were controlled by restriction during sample selection. But since it was a case-control study, it is still recommended that studies involving the use of active coping and behavior management strategies should be carried out to perceive and modulate the effect of psychological stress which is slowly and steadily becoming a worldwide health scare.

Limitations of the Study

Within its scope, this study postulated that stress can potentially cause worsening of the periodontitis. Since this was a case-control study so there were a few limitations of the study which included:

1. The data collected was dependent on the patient's recall capacity about the exposure to an untoward event which may have suffered "Recall bias".
2. Since it was an observational study, the effect of therapeutic strategies on this plausible association could not be established.
3. The temporal relationship between exposure (stress) and disease (periodontitis) could not be estimated.

Conclusion

With the current evidence, a very close interrelationship is evident in stress and periodontitis which involves dysregulation of immunoinflammatory responses with an increase in the deleterious oral microbiome. Further research exploring better assessment tools and synergistic approaches for managing stress and periodontitis is essentially demanded. Within the scope of this study and on the evaluation of the results obtained it was concluded that psychological stress may be positively associated with chronic periodontitis and thus stress and poorer lifestyle can be considered as modifiable potential risk profiles in periodontitis patients.

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