

STRESS, CORTISOL AND PERIODONTAL STATUS - A CROSS SECTIONAL STUDY

Dissertation submitted to

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In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

**BRANCH – II
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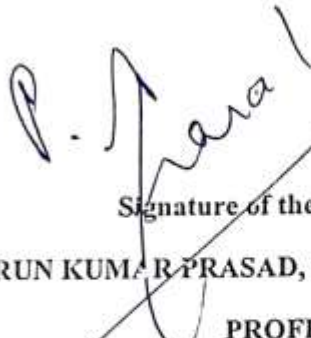
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ABBREVIATIONS

AA	Alpha amylase
Agp	Aggressive periodontitis
ANS	Autonomic nervous system
BOP	Bleeding on probing
b/w	between
CAL	Clinical attachment loss
CA	Chromogranin A
DHEA	Dehydroepiandrosterone
ELISA	Enzyme- linked immunosorbent assay
GCF	Gingival crevicular fluid
h/d	Hours per day
HPA	Hypothalamus –Pituitary-adrenal axis
IL	Interleukin
IgA	Immunoglobulin
Mins	Minutes
mm	Millimeter
ng	Nanogram
nmol	Nanomoles per liter
OHI	Oral hygiene index
OD	Optical density
P.gingivalis	Porphyromonas gingivalis

ABBREVIATIONS

SRP	Scaling and root planing
SRRS	Social readjustment rating scale
SD	Standard deviation
Th-1	T-helper -1
Th-2	T-helper-2
TMB	Tetra methylene benidine
VIP	Vasoactive intestinal peptide

INTRODUCTION

Stress is an unpleasant emotion which is faced in day to day life, the term stress is a Latin word which means “strained”, the concept of stress was introduced by Hans Selye in 1936, in layman term stress is an unpleasant emotion, the stimuli which lead to the stressful condition is called stressor, this stressor is broadly classified into major stressor and minor stressor. Many studies have stated that stress can lead to a change in the immune response through neural and endocrine pathways.¹

The stress is of 2 types namely acute stress and chronic stress, acute stress is associated with temporary response to the stressor, chronic stress results from continuous exposure to the stressor, as the name indicate acute stress usually last for few minutes to hours. In acute phase the body responds to stress by some infections, whereas in chronic condition it last from several hours even to months, where body responds to stress by altering the immune response, which leads to systemic diseases such as Diabetics Mellitus, Hypertension etc.^{1,2}

When there is a stressfull condition the pathway being activated is the “stress-hypothalamus-pituitary adrenal cortex” pathway, the hormone which is secreted in this condition is cortisol. Cortisol is a glucocorticoid which is secreted by the adrenal cortex.³

Whenever the individual faces a stressful condition the central nervous system signals hypothalamus to release corticotrophin and arginine vasopressin, which leads to the activation of adrenal cortex and results in release of steroid hormone, these hormone further reduce the activity of macrophages, lymphocytes, monocytes, neutrophils, and cytokines, which also alter the immune response by inhibiting antibody production.^{2,4}

Cortisol a corticosteroid hormone, usually follows a circadian rhythm or a biological clock, the concentration of cortisol is usually increased in morning and reduces in evening, the normal serum salivary cortisol level is about 10-20microns/deciliter and the salivary cortisol level ranges from 0.4-3.6nmol/lit, the concentration of cortisol is usually elevated in psychiatric individuals and Cushings disease.^{2,3}

Periodontal disease is a chronic inflammatory disease, which is complex and multifactorial, periodontitis consists of many risk factors one of that is stress⁶, though the exact mechanism of how stress affects the periodontium is not known, and it has some influence on periodontal structure¹. In stress the autonomic nervous system plays an important role on salivary gland by releasing salivary alpha amylase, which is an important component of mucosal immunity, i.e. which inhibits bacterial adhesion, aggregation, and growth.

ANS also regulates substance -p neuro-peptide a pro inflammatory cytokine, in stress condition, there is an alteration of immune responses and increase in the composition of pathological bacteria thus stress impair the ratio of pro inflammatory and anti inflammatory mediators and leads to periodontitis. Apart from this, cortisol can reduce the saliva secretion and the salivary pH which leads to accumulation of plaque, and further neglect of oral hygiene leads to periodontitis.

Immunologically there is change in t-helper 1 and t-helper 2 ratio, where the t-helper 2 ratio increase leads to alteration of B-cell dysfunction and humoral activity, the healing mechanism of the oral cavity is also altered due to increase production of catecholamine's, which leads to reduced oxygen supply due to increased vasoconstriction, which inhibits angiogenesis and epithelisation.⁵

Thus the aim of my study is to assess the relationship between Stress and Periodontitis.

AIMS AND OBJECTIVES

- Evaluate the salivary cortisol level in periodontally healthy, gingivitis and chronic periodontitis who are all systemically healthy.
- To investigate and compare the presence of salivary cortisol levels among the three groups.
- To assess the correlation of salivary cortisol levels and anxiety sum among the three groups.

REVIEW OF LITERATURE

Periodontitis is a chronic inflammatory disease in which, the host and environment plays a major role, there are many risk factor associated with periodontitis one among that is stress⁶. According to Lazarus stress is a “An inharmonious fit between the person and the environment, one in which the person’s resources are taxed or exceeded, forcing the person to struggle, usually in complex ways, to cope this, stress can alter the body homeostasis. Hence stress is triggering factor for many disease condition.⁷

Stress can be due to certain life situation or due to external environment, the stimuli which induce stress is called stressor.¹ It can be either be acute or chronic, chronic stressor leads to affect the individual both physically and mentally so the basic symptoms of stress are poor maintenance of oneself, mostly avoid social contacts, prefer to be alone, tooth grinding, overeating or loss of appetite.⁴

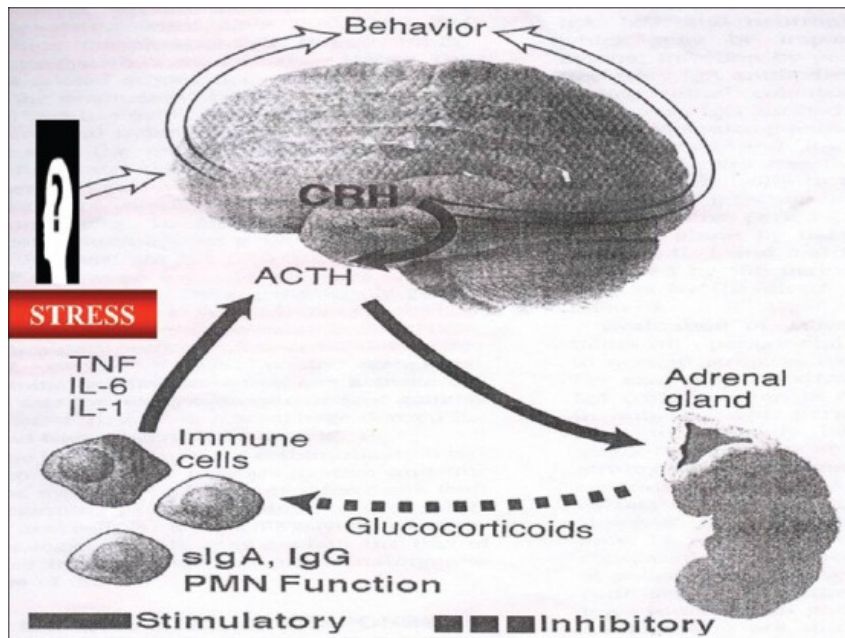


Figure.1: Mechanism of stress

The main hormone which is released during stress is cortisol. Cortisol is released from adrenal cortex, the ANS plays a vital role in response to stress, so whenever in a dangerous situation or stress full condition there is a release of fright and flight response from the brain, then the Hypothalamus pituitary-adrenal axis further mediate the response by releasing the corticotrophin-releasing hormone, arginine and vasopressin that acts on the pituitary gland. Pituitary gland releases the adrenocortical hormone, which acts on adrenal cortex, which further release and increase the secretion of cortisol which is a glucocorticoid a steroid hormone.¹

Many studies stated that stress impairs immune response by reducing the body's inflammatory response, by increasing blood glucose level and release of inflammatory mediators, stress plays a major role in altering the immune response.²

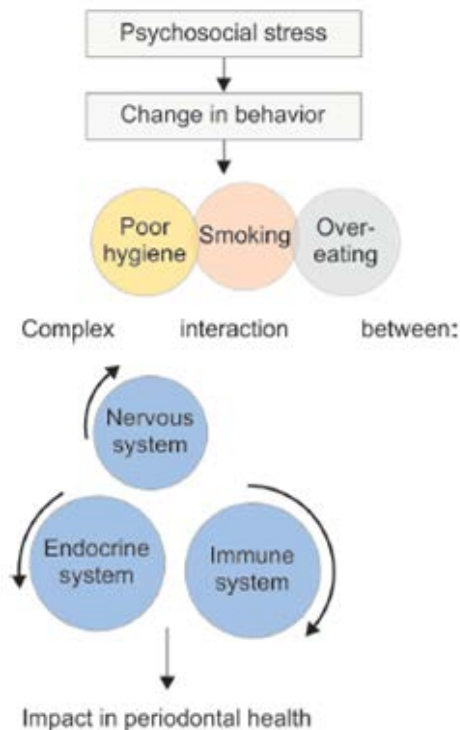


Figure.2: Stress impact on periodontal health

So whenever an individual is disoriented or highly impaired, he poses poor oral hygiene and mostly develops a habit of consumption of alcohol, smoking and poor nutrition intake, which makes an individual easily prone to periodontitis. When there is a chronic stress condition it alters the immune response by release of more pro-inflammatory cytokines, thus stress impairs the pro-inflammatory and anti-inflammatory cytokines, which leads to alteration in interleukin -1, 6 and prostaglandins which makes the individual susceptible to periodontitis.³

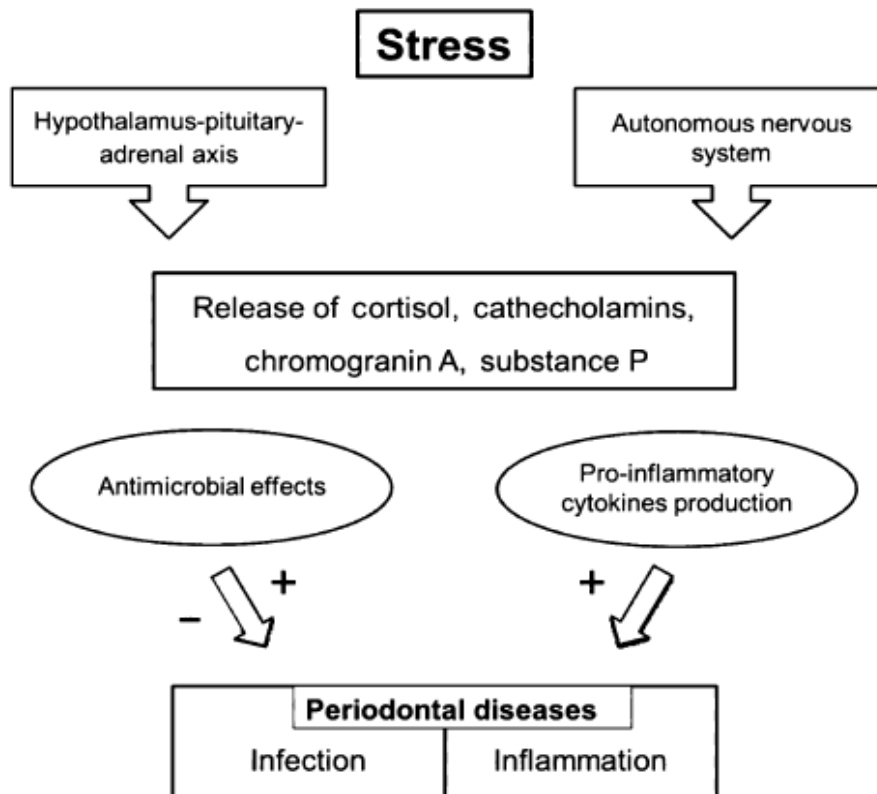


Figure.3: Action of stress on inflammatory mediators

Stress also causes changes in the salivary flow in the oral cavity which leads to change in salivary pH and reduces the activity of antibacterial substances in saliva such

as mucin, agglutinins, lysozyme, lactoferrin, salivary peroxidases, and histidine-rich peptides.

These help to protect the oral cavity from bacterial invasions and colonization that leads to an increase in the production of plaque and calculus and aids in the pathway to periodontitis.

The immune response is triggered from the gingival tissue, the cytokines are released from the immune cells which release matrix metalloproteinase a zinc-dependent endopeptidase, this cleaves extracellular matrix, followed by fibroblast degradation and bone resorption.

The immune response in periodontitis act as a double-edged sword, the protective mechanism of the immune system which further leads to the destruction of the periodontal structures and stress aggravates this condition.

In stress, there is suppressed action of interleukin 2 and T –cell mediators and NK cell functions. The T helper cell is of 2 types they are classified depending upon cytokine profile Th1(T-helper 1) and Th2 (T-helper 2). Th1 produces cytokines like IL-2 and interferon and Th2 produces IL -4, IL-5, IL-6 and IL -10, in case of stress condition there is dominance of Th2 cells which is observed as in a stressfull condition and this mechanism further leads to degradation of periodontal structures.⁸

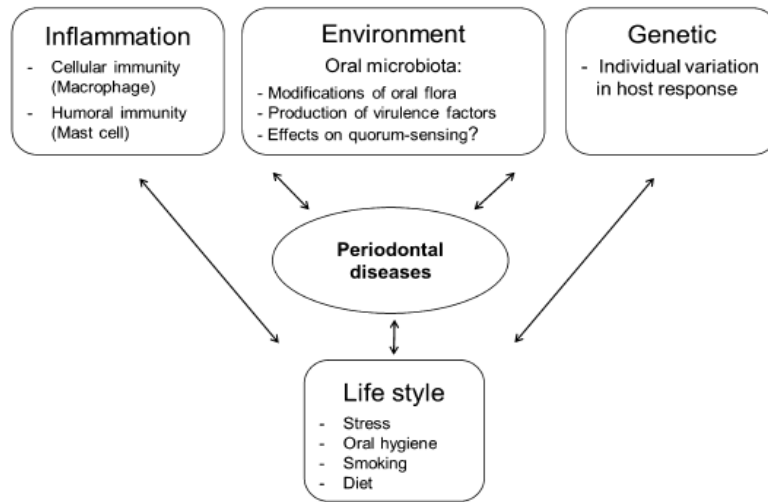


Figure: 4 Relationship between stress and periodontal disease

Apart from immune response, neglect of oral habits, smoking, and other habits frequency will increase during stress full condition, which may lead to periodontitis.⁸ Thus stress can alter the immunological neurological and endocrine balance in an individual, which aid in periodontal destruction and other disease manifestation.⁸

REVIEW OF LITERATURE

Freeman et al (1993)¹⁰ conducted a study to investigate an occupational stress upon periodontal health. 18 individuals were selected for the study, all clinical parameters were measured in their baseline visit and subsequent occupational stress including clinical parameters was recorded for 12 months. The occupational stress was evaluated by occupation stress indicator, there was notable increase in probing depth in individuals with high occupational stress, and the author concludes that periodontitis may be associated with psychological factors.

Deinzer et al (1998)¹¹ conducted a study to evaluate the relationship between stress and its effect on oral hygiene. 51 students were selected for the study, 29 were exam goers and 22 are non-exam goers students, all clinical parameters were measured. 4 days before the onset of the first exam the saliva sample was collected from both the students groups and a second sample was collected from the exam goers and non-exam goers on the last day of their exam and psychological questionnaire was asked to be answered by the students based on frequency of oral hygiene during their exam days. The result stated that the oral hygiene frequency was worse in exam going students when compared to non-exam going students, similarly salivary cortisol level was high in exam goers.

Deinzer et al (2001)¹² conducted a study to evaluate the academic stress on periodontal health. 32 medical students were selected for the study, clinical parameters such as bleeding on probing, clinical attachment loss, probing depth and gingival index, plaque index were measured . On the day 1 of the study

prophylaxis was done on the last day of exam, questionnaire regarding oral hygiene was answered by the individuals. The author concluded that psychological stress has an impact on periodontal status, where exam stress leads to poor oral hygiene and increased plaque accumulation.

Hugosun et al (2002)¹³ conducted a study, to investigate whether negative life event leads to increase periodontal disease. 298 individuals aged 50 - 80 years participated in the study, radiographic investigation were made, all clinical parameters were measured, followed by questionnaire regarding socioeconomic status, the result of the study was life events, being one of the risk factor psychological breakdowns may also lead to periodontal disease.

Mengel et al (2002)¹⁴ conducted a study is to evaluate the level of interleukin 6, interleukin-1 β and cortisol level in peripheral blood of periodontitis patients and its interaction with psychological stress. 30 patients were selected for the study which consist of aggressive periodontitis and generalized chronic periodontitis. All clinical parameters were measured, individuals were asked to answer the questionnaire of stress and smoking. The saliva sample was collected in the morning and analyzed using ELISA. The result stated that there is an increase in interleukin 1 β , IL 6, IL beta and salivary cortisol level in aggressive periodontitis patients.

Nayak et al (2003)¹⁵ conducted a study to asses, the association of cortisol level between anxious and non-anxious chronic periodontitis individuals. 45 individuals were selected for the study and divided into 3 groups, the group-1 consist of healthy individuals, group-2 of chronic periodontitis with no anxiety

and group-3 consist of chronic periodontitis with anxiety, all clinical parameters were recorded and saliva samples were collected and estimated using ELISA, the psychological evaluation was made by answering the STAI –inventory scale, this study concluded that there is an increasing level of salivary cortisol in individuals of chronic periodontitis with anxiety.

Saletu et al (2005)¹⁶ conducted a study to assess the link between depression and development of periodontitis. 41 individuals were selected for the study all clinical parameters were evaluated, Hamilton Depression Scale, the Zung Self-Rating Depression and Anxiety Scales, the von Zerssen Well-being and Complaint Scales, the Epworth Sleepiness Scale, the Pittsburgh Sleep Quality Index, the Quality-of-Life all these questionnaires were answered by all the participants, after analysis of the clinical parameters and the questionnaire, it states that there is a connection between depressive mood and periodontitis.

Johannsen et al (2006)¹⁷ conducted a study to evaluate the relationship of oral health status, pro-inflammatory markers and cortisol levels in GCF and saliva in patients with depression and its effect on periodontitis. 43 women were taken in to the study and 29 were allotted as control, all clinical parameters were measured. Interleukin (IL)-1b, IL-6, and matrix metalloproteinase were detected with enzyme-linked immunosorbent assay and cortisol with radioimmunoassay, individual with stress-related depression had an increase cortisol, GCF levels and IL6.

Castro et al (2006)¹⁸ conducted a study, where he investigated, the relationship between anxiety, depression life events and periodontitis. 165 individuals were selected for the study and divided into 2 groups, 96 participants in case group and 69 in control

group. The STAI inventory scale and Beck scale were answered among the participants and the answers were analyzed, the author concluded that there was a strong association found between psychological status and periodontitis.

Hugo et al (2006)¹⁹ conducted a study to evaluate the relationship between stress, depression and cortisol levels in individuals aged more than 50 years. 230 individuals were selected for the study, all clinical parameters were measured, the Becks inventory scale was answered by all the individuals, the saliva samples were collected in 3 intervals before breakfast, before lunch and before dinner. All saliva samples were centrifuged, the samples were analyzed with ELISA and was compared with plaque index and gingival index. The result of this study stated that Cortisol values, plaque index, and gingival index were significantly correlated.

Hilgert et al (2006)²⁰ conducted a study to evaluate the relationship between salivary cortisol and its association between the severity of periodontitis in elderly individuals aged 50 years and more. 235 subjects were selected for the study all clinical parameters were evaluated, the psychological analysis was, made using LIPPS stress symptoms adult inventory scale. Saliva sample was collected in the morning between 8 am to 9 am, the second sample was collected between 11 am to 1 pm and the third sample was collected between 8 pm to 9 pm. The samples were analyzed and the mean of the questionnaire was done, the result of this study stated that high-level salivary cortisol in older individuals was associated with periodontitis.

Nakajima et al (2006)²¹ conducted a study to assess the effect of restraining strain and periodontal condition. 21 male rats were taken for the study and divided into 4 groups. Group A (controls), group B (exposed to restraint stress for 12 h/d for 22 d),

group C (orally challenged with *P. gingivalis*), and group D (exposed to restraint stress for 12 h/d for 22 d and orally challenged with *P. gingivalis*). After 22 days all animals were killed, after that the bone level was measured, the concentration of adenocorticoid was measured and result stated that the restrain strain enhances the progression of periodontitis with increased *Prophyromonas gingivalis* bacterial count.

Ishisaka et al (2008)²² conducted a study to find the association between the levels of dehydroepicortisone sulfate and cortisol levels in older individuals. 467 samples were selected for the study all clinical parameters were measured, blood samples were collected questionnaires were answered by all individuals. The author concluded that cortisol had an association between periodontitis, where no association was found between DHEA and periodontitis.

Rosania (2009)²³ conducted a study to explore the association between psychological factors, periodontal disease and psycho neuroimmunological variables. 45 individuals were selected for the study, who underwent a non-surgical periodontal therapy, while some were in maintenance phase after surgical intervention. All clinical parameters were measured, the health and oral hygiene survey was carried out in questionnaire form among the participants and the saliva samples were collected in a passive drooling manner from all the individuals for salivary cortisol analysis using ELISA. This study concluded that patient with more missing teeth, mobile tooth had more stress score and increased salivary cortisol level.

Ansai et al (2009)²⁴ conducted a study to evaluate the relationship between Cortisol and DHEA levels in elderly individuals, 171 subjects were selected for the study, who are all systemically healthy, both smoker and nonsmokers were included in

the study. All clinical parameters were measured, verbal questions regarding oral habits and lifestyle was asked using Face scale and scored, the saliva samples were collected and estimated. The result concluded that Cortisol and DHEA level were high in smokers when compared to nonsmokers and cortisol can be used as a biomarker for periodontitis.

Rai et al (2011)²⁵ conducted a study to explore the relation between periodontal disease, psychological factor, salivary stress and health behavior. 100 individuals were selected for the study, all clinical parameters was measured SRP was done. If in need of any surgical intervention it was carried out during a clinical course of 4 months. A stress profile questionnaire was answered by the participants, saliva samples were collected from all and the samples were analyzed using ELISA and amylase kit, the result concluded that increase in the level of salivary markers was associated with periodontitis.

Mahendran et al (2011)²⁶ conducted a study to evaluate the relationship between salivary cortisol and chronic periodontitis in Tamilnadu patrol of Cuddalore district. 110 individuals were selected for the study and were divided into 2 groups. The test had probing depth more than 6mm and control group had probing depth less than 5mm. Psychological evaluations were done using a questionnaire. Saliva samples were collected and the analysis was made using ELISA, the result stated that the mean of occupational stress and salivary cortisol was increased in test group when compared to control group.

Haririan et al (2012)²⁷ et al conducted a study to assess the relationship between chromogranin A and alpha-amylase (AA) in saliva and serum of disease and health and

its association with periodontitis. 88 individuals were selected for the study and divided into 3 groups, the Agp, the chronic periodontitis and healthy, all clinical parameters were, measured the subjects were asked not to drink, smoke and eat from midnight till sample collection. The sample collection was done between 8 am to 11 am the saliva and blood sample were centrifuged and stored in deep freezer until the day of analysis. Later analyzed using Elisa kit, the study concluded that higher level of CA and cortisol was associated with aggressive periodontitis group.

Vettore et al (2013)²⁸ conducted a study to assess the relationship between stress and anxiety, measured by psychometric instruments, along with clinical parameters, in patients with different levels of periodontitis. 79 individuals were selected for the study and divided into two groups depending upon probing pocket depth, where the first group is taken as control and the second group as test, all clinical parameters were measured, the psychometric analysis was done using stress symptom inventory and SRRS. On comparing with the results of these groups individuals with high level of probing depth had a positive correlation with the questionnaires.

Refugio et al (2013)²⁹ conducted a study to evaluate the relationship between stress, salivary cortisol, and chronic periodontitis, 70 systemically healthy individuals were selected for the study, all clinical parameters such as BOP, CAL, Probing pocket depth and mobility were evaluated. The anxiety and depression scale was evaluated by using Zung scale and saliva sample was collected between 9 am to 11 am in the morning before brushing, eating and drinking, sample was analyzed using ELISA, the result of the study stated that out of 35 patients one from healthy group was diagnosed with depression and anxiety, in periodontitis group, there is a strong association between

salivary cortisol level and the psychoanalytical scale. Thus this study concludes that there is a strong association between stress and cortisol.

Agrawal et al (2014)³⁰ conducted a study to evaluate the relationship between psychological stress and chronic periodontitis. 60 patients were selected for the study and divided into 2 groups each group consist of 30 individuals with a mean age ranging from 30-35 years. All clinical parameters such as bleeding on probing, clinical attachment level and probing depth were measured. A questionnaire regarding socioeconomic status, smoking, health history and health problems were answered by all the participants. 1.5 ml venous blood was drawn between 9 am to 11 am and analyzed using enzyme-linked fluorescent assay, patients under chronic periodontitis was associated with increased serum cortisol level and showed a correlation between clinical parameters.

Ardila et al (2015)³¹ conducted a study to evaluate the association between periodontal pathogenesis and cortisol level in chronic periodontitis. 75 individuals were selected for the study and divided into mild, moderate and severe periodontitis groups. All clinical parameters were measured, sociodemographic questionnaire was answered by the individuals, the blood samples were collected, the microbiological samples were obtained from the deepest periodontal pocket. This study concluded that *P.gingivalis* count was more in individuals with increased serum cortisol level. The author concluded that high level of cortisol could increase the viability of *Porphyromonas gingivitis*.

Jaiswal et al (2016)³² conducted a study to assess the relationship between physical health, mental health and its effect on periodontal health. 40 individuals were selected for the study and divided into 2 groups each group consist of 20 individuals of healthy and chronic periodontitis subjects, all clinical parameters were measured the

individuals with probing depth less than 3mm and no clinical attachment loss was grouped under healthy category and individuals with probing depth more than 3mm and clinical attachment loss, was grouped under periodontitis, blood samples were collected between 9 am to 11 am by vein puncture to estimate the serum cortisol level and a psychological questionnaire by Cohan in 1983, was answered by all individuals and sample was analyzed with ELISA kit, the results stated that increase in serum cortisol value and high psycho analytical scale mean was associated with the periodontitis group.

Cakmak et al (2016)³³ conducted a study to estimate the level of cortisol and dehydrocortisone in gingival crevicular fluid and saliva, in generalized chronic periodontitis, aggressive chronic periodontitis and periodontally healthy individuals. 92 individuals were selected for the study, all clinical parameters such as bleeding on probing, clinical attachment loss, probing pocket depth and plaque index were measured. For aggressive periodontitis group, the inclusion criteria was probing depth should be more than 6mm, followed by severe bone loss, for chronic periodontitis, bleeding on probing was more the 50%, clinical attachment loss and probing pocket depth should be equal to 5mm, for healthy no signs of bone loss and periodontitis should be noted, GCF and saliva samples was collected from the individuals with fasting and without brushing around 8 am to 9 am. The sample was evaluated with ELISA and concluded that there is increased level of salivary cortisol and dehydrocortisone in aggressive periodontitis patients when compared to chronic periodontitis and healthy individuals.

Fenol et al (2017)³⁴ conducted a study to evaluate the association between stress, salivary Cortisol and periodontitis among the inmates of prison.70 individuals were selected for the study and divided into 2 groups based on periodontal probing depth. The

group- A had individuals with probing depth greater than 4mm and lesser than 6mm, the group –B had a probing depth, greater than 6mm, the clinical parameters such as gingival index, clinical attachment loss, and OHI was measured, the stress level was evaluated using depression stress and anxiety scale. The salivary samples were collected 2 hours before brushing and eating, and the author stated that individual with more probing depth were associated with high stress and concluded that Cortisol is a stress biomarker which is associated with periodontitis.

Haririan et al (2018)³⁵ conducted a study to investigate, the presence of neuropeptides and cortisol in saliva and serum in periodontal health and disease, 100 individuals were selected for the study, 56 periodontitis, 21 aggressive periodontitis, 44 periodontally healthy subjects, all clinical parameters were evaluated, the psychology stress was assessed with Wearness scale and Warning scale, the saliva and the blood samples were collected and tested for neuropeptides and cortisol. The neuropeptides such as VIP and cortisol was high in saliva when compared with serum, for patients with aggressive and chronic periodontitis.

Penmetsa et al (2019)³⁶ conducted a study to evaluate the correlation between stress, anxiety and periodontitis among health care professional students. 375 medical science related students were selected for the study, which comprises 125 medical students, 125 dental students and 125 pharmacy students all clinical parameters were measured, the depression anxiety questionnaire was asked to answer by all the participants and the mean of the answers were calculated, the results obtained indicated that the stress level was high in medical students when compared to dental and pharmacy students.

Mannem et al (2019)³⁷ conducted a study to find the association between psychological stress and chronic periodontitis, 111 individuals were selected for the study, with a mean age of 45 years all clinical parameters such as bleeding on probing, clinical attachment loss, probing pocket depth were evaluated, saliva samples were collected on the next day morning between 8 am to 10 am and the LIPPS psychological questionnaire was answered by all the participants. The samples were analyzed, and the individual who had high mean score had increased cortisol level and was associated with periodontitis.

Sundararajan et al (2019)³⁸ conducted a study to ruin out the relationship between stress and periodontitis, 35 individuals were selected for the study all clinical parameters were measured and the individuals were asked to answer the Becks questionnaire, and the individuals with high probing depth and clinical attachment level had a positive correlation with questionnaire, thus the study states that stress is associated with periodontitis.

Mudrika et al (2019)³⁹ the aim of the study was to find the relationship between cortisol, DHEA levels in periodontitis and healthy subjects, 20 individuals with an age group ranging from 35-60 years was enrolled in this study who all are systemically healthy, all clinical parameters such as bleeding on probing, clinical attachment level, probing pocket depth were measured, the saliva and gingival crevicular fluid samples were collected in fasting, between 9 am to 11 am. Samples were analyzed, the result stated that there is an association between salivary cortisol and DHEA in periodontitis group, there is an increase in salivary cortisol and DHEA levels in periodontitis group when compared to healthy group.

Goyal et al (2019)⁴⁰ conducted a study to find out the relationship between psychological stress and periodontitis. 47 individuals were selected for the study, SRRS questionnaire was answered by the individuals all clinical parameters were measured, The group1 consist of 16 chronic periodontitis individuals and the group-2 consist of 31 stressed subjects, and the blood samples were drawn and analyzed for both the groups and the result stated that group1 is significantly positive and group2 correlates with the mean value of the questionnaire and serum cortisol level.

MATERIALS AND METHODS

PATIENT SELECTION:

The sample included 90 patients selected from the outpatient ward, Department of Periodontology, K.S.R institute of dental science and research, Tiruchengode, Nammakal district, Tamilnadu. The study protocol was explained to the patients and written informed consent and institutional ethical clearance were obtained.

INCLUSION CRITERIA:

- Systemically healthy individuals diagnosed with generalized chronic periodontitis (male and female).
- Systemically healthy individuals diagnosed with generalized chronic gingivitis.
- Systemically healthy and periodontally healthy individuals.

EXCLUSION CRITERIA:

- Pregnant and lactating women.
- Patients with the systemic disorder.
- Patients under any immune-suppressive drugs.
- Patient under corticosteroids.
- Patients under antibiotics before 6 months.
- Patient under any periodontal therapy.

STUDY DESIGN:

The study sample consists of 90 individuals; they are divided into 3 groups:

- Group 1: Healthy
- Group 2: Gingivitis
- Group3: Chronic periodontitis

The first group included 30 individuals who were systemically and periodontally healthy, the second group includes 30 members who were systemically healthy with generalized chronic gingivitis, the third group consists of 30 members who were systemically healthy with chronic periodontitis. The clinical parameters such as plaque index, gingival index, probing depth and STAI inventory scale were recorded. The saliva sample was collected from all the subjects before non-surgical periodontal therapy.

The levels of cortisol in the saliva sample were analyzed by using a direct enzyme immunosorbent assay.

ARMAMENTARIUM FOR IN VIVO EXAMINATION:

- Mouth mirror.
- William's graduated periodontal probe.
- Dental explorer.
- Cotton plier.
- Cotton roll.
- Stainless steel tray.
- Gloves and face mask.

ARMAMENTARIUM FOR UNSTIMULATED SALIVA COLLECTION:

- Plastic vials.
- Thermocol box.
- Dry ice.

CLINICAL PARAMETERS:

1. Plaque index-Loe's modification (1967)
2. Gingival index-Loe's modification (1967)
3. Probing pocket depth

PLAQUE INDEX-(PI):

The plaque index was described by SILLNESS and LOE. H in 1964 and was modified by LOE. H in 1967. The plaque was assessed using a mouth mirror and dental explorer after air-drying the teeth to assess plaque on different areas namely mesiofacial, facial, distofacial and lingual surface.

INSTRUMENTS USED:

- Mouth mirror and a dental explorer.

The teeth are air-dried and examined visually; when no plaque was visible an explorer was used on the surface. The explorer was passed across the surface in the cervical third and near the entrance to the gingival sulcus. The following scores were given.

SCORES FOR PLAQUE INDEX:

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe, across the tooth surface
2	Moderate accumulation of soft deposit in gingival pocket on gingival margin on or adjacent to tooth surface, which can be seen in naked eye
3	Abundance of soft matter within the gingival pocket and /or on the gingival margin and adjacent tooth surface

CALCULATION OF PI:

PI score for the area:

Each area (disto facial, facial, mesio- facial, lingual) is assigned a score from 0 to 3

PI score for a tooth:

The score from the four areas of the tooth are added and then divided by four.

PI score for the individual:

The indices for each tooth are added and then divided by a total number of teeth examined.

The score range from 0 to 3.

INTERPRETATION:

Excellent	0
Good	0.1-0.9
Fair	1-1.9
Poor	2-3

GINGIVAL INDEX:

The gingival index was developed by LOE.H and SILNESS. J in 1963, solely to assess the severity of gingivitis and its location in four possible areas by examining only the qualitative changes ie .., the severity of the lesion of the gingival soft tissues, in 1967 LOE detailed the sequence of examination to include entire teeth instead of the index teeth.

INSTRUMENTS USED:

- Mouth mirror and periodontal probe

The tissue surrounding each tooth was divided into four gingival scoring units. The mesiofacial papilla, facial margin, disto facial papilla, and entire lingual gingival margin. The teeth and the gingiva should be dried lightly with a blast of air or cotton roll. Each of the four gingival units was assessed and the following scores were given.

SCORE FOR GINGIVAL INDEX

Score	Criteria
0	Absence of inflammation/normal gingiva.
1	Mild inflammation, slight change in colour, slight edema, no bleeding on probing.
2	Mild inflammation: moderate glazing, redness, edema, and hypertrophy, bleeding on probing.
3	Severe inflammation: marked redness and hypertrophy.

CALCULATION OF GI:

GI score for the area:

Each area facial, facial, mesio facial, lingual is assigned as a score from 0 to 3.

GI score for a tooth:

The score of the four areas of the tooth are added and then divided by four.

GI score for the individual:

The indices for each tooth are added and then divided by the total number of the teeth examined. The score ranges from 0 to 3.

INTERPRETATION:

Gingival score	Condition
0.0-1.0	Mild gingivitis
1.1-2.0	Moderate gingivitis
2.1-3.0	Severe gingivitis

PROBING POCKET DEPTH:

Probing pocket depth was measured as the distance between the free gingival margin and the base of the pocket.

INSTRUMENTS USED:

- Mouth mirror and Williams periodontal probe.

CLINICAL ATTACHMENT LEVEL (CAL):

The clinical attachment level was measured as the distance from the CEJ to the base of the pocket.

STAI INVENTORY SCALE:

It is a psychoanalytic scale composed of 40 questions on the basics of how they feel about themselves on daily routine and 4 choices had been given for them to choose.

SALIVA SAMPLING:

A saliva sample was collected according to the unstimulated saliva collection procedure. The individuals were asked to report to the department for saliva collection without brushing and breakfast. All saliva samples were collected between 9:00 am to 9:30 am. Patients were asked to sit comfortably with head tilted forward and were asked to swallow their saliva first and then to allow the saliva to drain passively for 10-15 mins over

the lower lip into the polypropylene tube before the clinical measurements. After that the saliva samples were centrifuged at 2500-3000 rpm, for 20 mins, the supernatant is collected, transferred to another plastic tube and stored in -20 degree Celsius until the day of analysis.

STAI inventory scale for anxiety, a questionnaire was answered by all the individuals to assess their anxiety levels.

ASSAY PROCEDURE:

The cortisol level in the collected saliva samples was assessed using an enzyme-linked immunosorbent assay (ELISA) kit. (Human salivary cortisol kit: DBC co.ltd, Canada).

The assay was a quantitative ELISA technique. ELISA is one of the immunoassay methods using an antibody to capture an antigen. The enzyme-labeled antibody to estimate the amount of antigen. The ELISA technique used in this study is a direct technique.

A Human salivary cortisol kit from the DBC technology laboratory was employed to analyze the cortisol level in saliva.

MATERIALS AND METHODS TO ASSESS CORTISOL LEVEL IN SALIVA BY ELISA:

- Precoated ELISA plate.
- 96-well cover sheet.
- Standard solution.
- Standard diluents.
- HRP conjugate reagent.

- Wash buffer concentrate.
- Chromogen solution A.
- Chromogen solution B.
- Stop solution 6ml.
- An incubator which can provide stable incubation conditions up to 37degree Celsius \pm 0.5 degrees Celsius.
- Micro plate reader capable of measuring absorbance at 450nm.
- Precision pipettes delivery 2 μ ml to 1 ml volumes.
- 100 ml and 1 lit graduated cylinder.
- Absorbent paper. Distilled /deionized water.
- Log-log graph paper for computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.
- Container for wash solution.

ASSAY PROCEDURE:

The stored samples were defreezed by placing the samples at room temperature, then the samples were duplicated and all the procedure was performed in 2 different kits. All reagents were brought to room temperature 18-25 degree Celsius.

REAGENT PREPARATION:

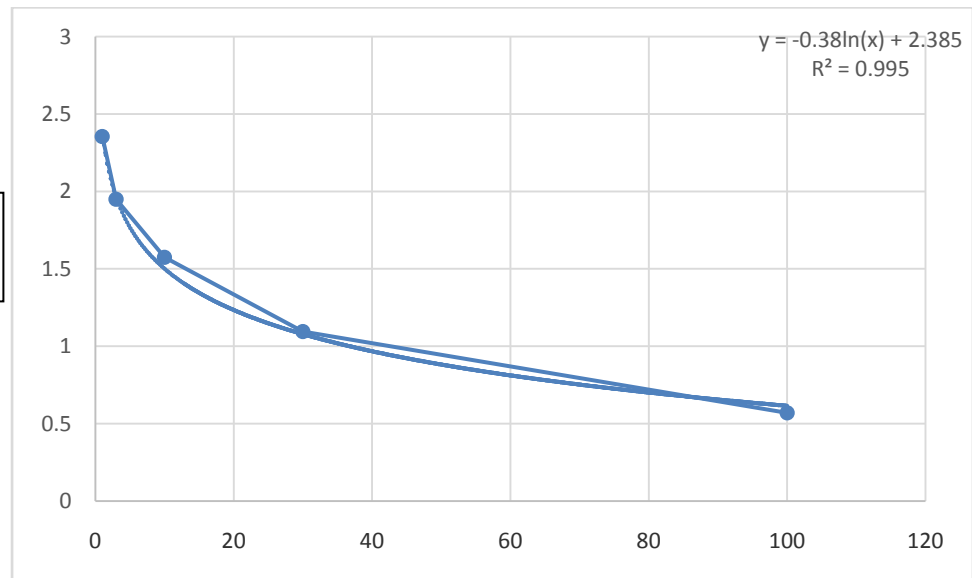
- Preparation of standard: The 10 standard wells on the ELISA were coated and labeled 1 to 10. First, standard 100 μ l and then the standard diluents 50 μ l was added to first and second well and mixed and 100 μ l from the above wells were taken out and added to the third and fourth well separated and then standard diluents 50 μ l was added to third and fourth well and mixed, 50 μ l taken out from third and fourth well and discarded, again 50 μ l was taken out from third and fourth well and added to fifth and sixth well.

Standard diluent 50 μ l was added to the fifth and sixth well and mixed. 50 μ l was taken out from the fifth and sixth well and added to the seventh and eighth well and standard diluents. 50 μ l was added to the seventh and eighth well and mixed, again take out 50 μ l from the seventh and eighth well and added to the ninth and tenth well. Standard diluents 50 μ l was added to the ninth and tenth well and mixed 50 μ l and the concentration was 9ng/ml, 6ng/ml, 3ng/ml, 1.5ng/ml, 0.75ng/ml respectively.

- Sample diluents 40 μ l was added to the testing sample well and later testing sample 10 μ l was added.
- Samples were incubated for 30 min at 37°C after closing the plate with the closure plate membrane.
- Washing buffer was prepared: 30 fold wash solution diluted with Distilled water until 600 ml.
- Addition of 100 μ l of the buffer solution followed by addition of HRP conjugate.
- After 30 minutes shaking wash is given for 3 times.
- Remove the required number of microwell strips.
- Pipette 50 μ l of each calibrator, control and specimen sample into corresponding labeled well in duplicate, again in pipette 100 μ l of the conjugate working solution into each well.
- Incubate on plate shaker for 45 mins at room temperature.
- Wash the wells three times with 300 μ l of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that is dry.
- Pipette 150 μ l of TMB substrate into each well at a timed interval.
- TMB chromogen solution A 50 μ l and TMB chromogen B 50 μ l was added to each well and gently mixed, care was taken to evade the light.

- Incubate on a plate shaker for 15- 20 mins room temperature.
- Pipette 50ul of stopping solution, into each well at the same time interval.
- Read the plate on the microwell plate reader at 450nm within 20 mins after addition of the stopping solution.
- If the OD exceeds the upper limit of detection,50nm filter is unavailable, a 405or 415nm filter can be a substitute.
- The standard curve was plotted on log-log graph paper and using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis.
- The best-fit straight line was drawn through the standard points. The equation was derived to convert the cortisol values from nm to ng/ml.

**Absorption of
Cortisol at 450nm**



Concentration of Cortisol

Graph.1: Analysis of concentration of Cortisol



Figure 5: Generalized Chronic Periodontitis



Figure 6: Generalized Chronic Gingivitis



Figure 7: Periodontally healthy



Figure 8: Armamentarium for saliva sample collection

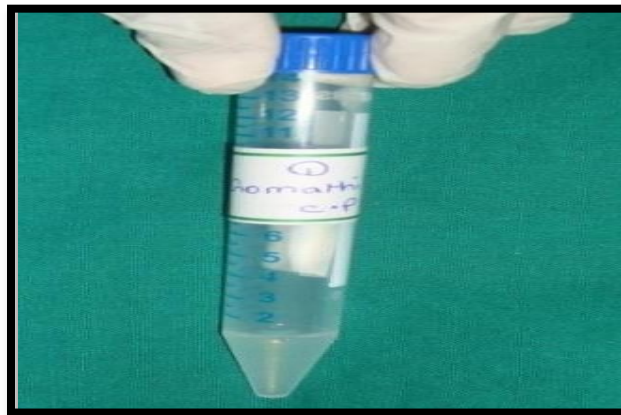


Figure 9: Collected saliva sample for centrifuge



Figure 10: Centrifuged salivary samples

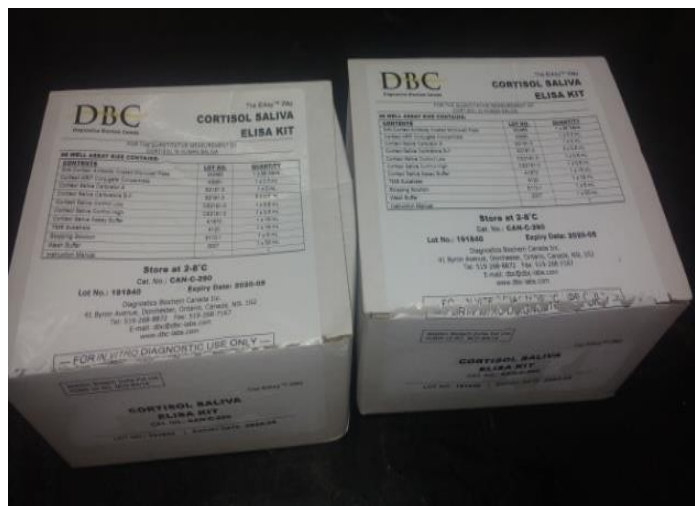


Figure 11: Human Salivary Cortisol kit



Figure 12: Un-boxed Salivary Cortisol kit and its reagents

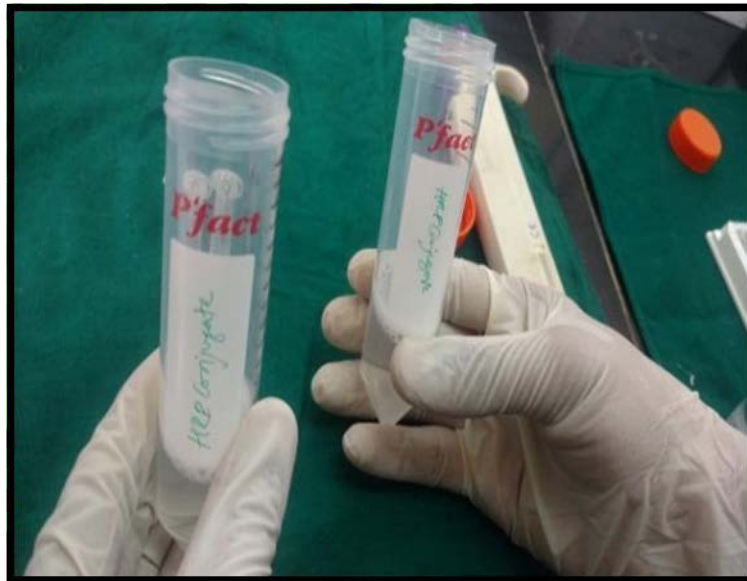


Figure 13: Preparation of HRP conjugate

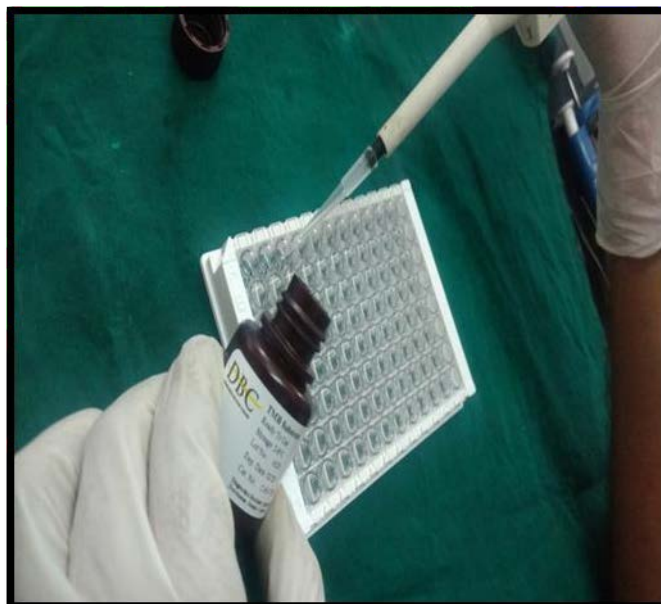


Figure 14: Analysis of sample



Figure 15: Placement of Elisa in a shaker



Figure 16: Elisa after processing

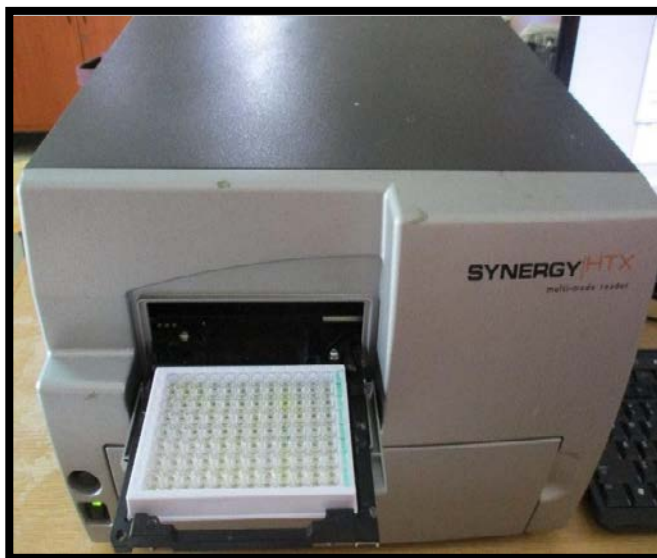


Figure 17: Elisa for reading analysis

STATISTICAL ANALYSIS

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. Released in 2013. Armonk, NY: IBM Corp., was used to perform statistical analyses.

Descriptive statistics:

The descriptive analysis includes expression of all explanatory and outcome parameters in terms of Mean & SD for continuous variables, whereas in terms of frequency and proportions for categorical variables.

Inferential Statistics:

One-way ANOVA test followed by Turkey's post hoc analysis was used to compare the mean anxiety sum scores and Cortisol levels between 3 study groups.

Pearson correlation test was used to assess the relationship b/w Anxiety sum scores & Cortisol levels in each study group.

Simple linear regression analysis was used to predict the Cortisol levels by Anxiety scores in each group.

The level of significance [P-Value] was set at $P < 0.05$.

RESULTS

Comparison of mean Anxiety sum scores between three study groups using One-way ANOVA Test.

Table 1, there is a comparison of three groups for the STAI questionnaire, which is used to predict the anxiety level of all individuals, the comparison of these groups was done using a one way- ANOVA test, when the mean and standard deviation of the group is found, the mean for group -1 (healthy group) has a value of 94.33 and the group -2 (gingivitis group) was 95.67 and for group-3 (chronic periodontitis), it was 102.57, and the p-value was kept at less than 0.005, from analyzing the standard p-value, the chronic periodontitis group had higher mean for anxiety score, the gingivitis had a moderate mean score when compared to periodontitis group and healthy group had least mean on comparing with other two groups.

Graph 2, there is an increase in the anxiety level of chronic periodontitis patients, when compared to gingivitis patients and there is a rise in the gingivitis anxiety sum graph, when compared to the healthy anxiety bar graph.

Comparison of mean Cortisol Levels (ng/ml) between three study groups using One-way ANOVA Test.

Table 2, there was a comparison of salivary cortisol values between the healthy group, gingivitis group and chronic periodontitis group. The analysis was done using one-way ANOVA test, the mean of the 3 groups are as follows, Mean 1.795 SD 0.313 for healthy subjects, Mean and SD for gingivitis was 3.959,0.528 and for chronic periodontitis group, Mean and SD was 4.355 and 0.322. On analyzing the above-mentioned values, the chronic periodontitis group had increased mean and SD value

when compared to other two groups, and showed a statistically significant result when compared between of gingivitis and healthy group.

Graph 3, represent the mean cortisol value. The chronic periodontitis group has higher mean salivary cortisol when compared to the other two groups.

Multiple comparisons of mean difference in Cortisol Levels (ng/ml) b/w three study groups using Tukey's post hoc Analysis.

Table 3, comparison between the mean cortisol values of healthy, gingivitis and periodontitis groups was done by using a turkey's post hoc analysis. On comparing the mean value between group-1 (healthy) and group-2 (gingivitis), the value was -2.164 and the p-value was less than 0.001, which is statistically significant, and states that the mean cortisol levels of the gingivitis was moderately high when matched between the healthy group.

On comparing the mean values of healthy group and chronic periodontitis group, the value ranges from -2.559 on further comparison with higher and lower limits of cortisol differentiation, the p-value was less than 0.001, which is a statistically significant, and the analysis states that the mean value of salivary cortisol level of chronic periodontitis group was higher when compared to the healthy group samples.

On further analysis between group-2 (gingivitis) and group-3 (chronic periodontitis), the mean cortisol value between the groups was -0.396.

On further investigation with the p-value between the groups, it was found to be less than 0.001, which states that the salivary cortisol level was high in chronic periodontitis group when compared to the gingivitis group.

Multiple comparisons of mean difference in anxiety sum scores b/w three study groups using Tukey's post hoc Analysis.

In table 4, comparison was made between the mean levels in the anxiety scale of healthy, gingivitis and periodontitis groups. On comparing the mean values of healthy and gingivitis groups, the p-value was 0.82 which was not significant, but on comparing the mean value of anxiety scale between healthy and periodontitis groups, the p-value was less than 0.001 which states that the anxiety sum score was highly significant to the chronic periodontitis group.

Similarly, on comparing the mean values of anxiety between group-2 and group-3 the p-value was less than 0.007, which is statistically significant and overall, the table states that the anxiety mean value for chronic periodontitis was higher when compared between other 2 groups.

Pearson correlation test to assess the relationship b/w Anxiety sum scores & Cortisol levels in each study group.

Table 5, sum of the anxiety scale and Cortisol values in all three groups are correlated and compared within the groups, the correlation was denoted as “r” in the table, for group -2 the correlation value is 0.42, which states that the anxiety score was moderately correlated with the cortisol value and on further analysis the p-value was 0.02, which states the relationship between Cortisol and anxiety scale is significant.

In group3 the correlation value of anxiety score and salivary Cortisol level is 0.65 and the p-value is less than 0.001 which implicates, the salivary Cortisol values and anxiety levels had a high correlation.

In group 1, the correlation value is 0.06, and the p-value is 0.72, which reveals that there was very little correlation between the anxiety level and salivary Cortisol values.

Simple linear regression analysis to predict the Cortisol levels by Anxiety scores in each group, in this table the anxiety.

Table 6, comparisons were done between anxiety score and Cortisol level among all the 3 groups and it was found that in group 3, there was a significant prediction of Cortisol levels by anxiety sum score, [$P < 0.001$], i.e. for every 1 score increase in Anxiety sum scores, the Cortisol levels increases, by 0.023 ng/ml at $R^2 = 0.56$.

Scatterplot depicting the relationship between anxiety sum core and Cortisol level in healthy, gingivitis and chronic disease.

Graph 4a, 4b and 4c representation of Cortisol values of the groups and its anxiety sum mean score was depicted. In healthy group, cortisol value range maximum of 2ng/ml, for gingivitis group the cortisol mean value ranges about 4 to 4.5 ng/ml and for chronic periodontitis group the mean cortisol value ranges more than 4.5-5 ng/ml with anxiety sum of all the three groups.

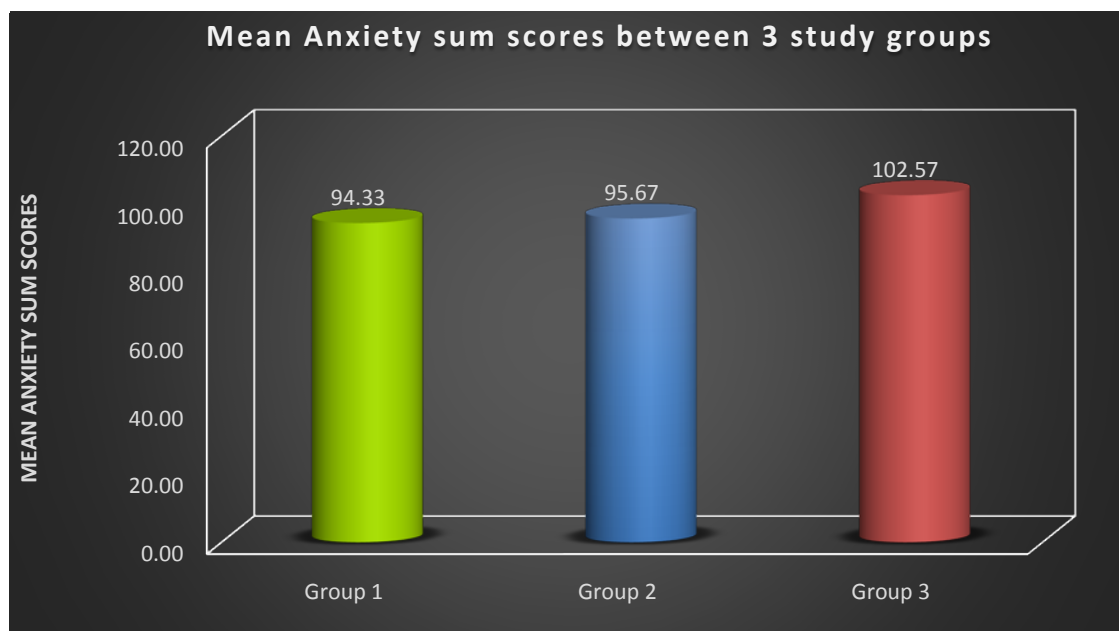
Comparison of mean Anxiety sum scores between three study groups using Oneway ANOVA Test.

TABLE: 1

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	30	94.33	8.03	81	112	0.001*
Group 2	30	95.67	6.89	80	109	0.001*
Group 3	30	102.57	10.30	75	117	0.001*

*Statistically Significant.

Graph: 2



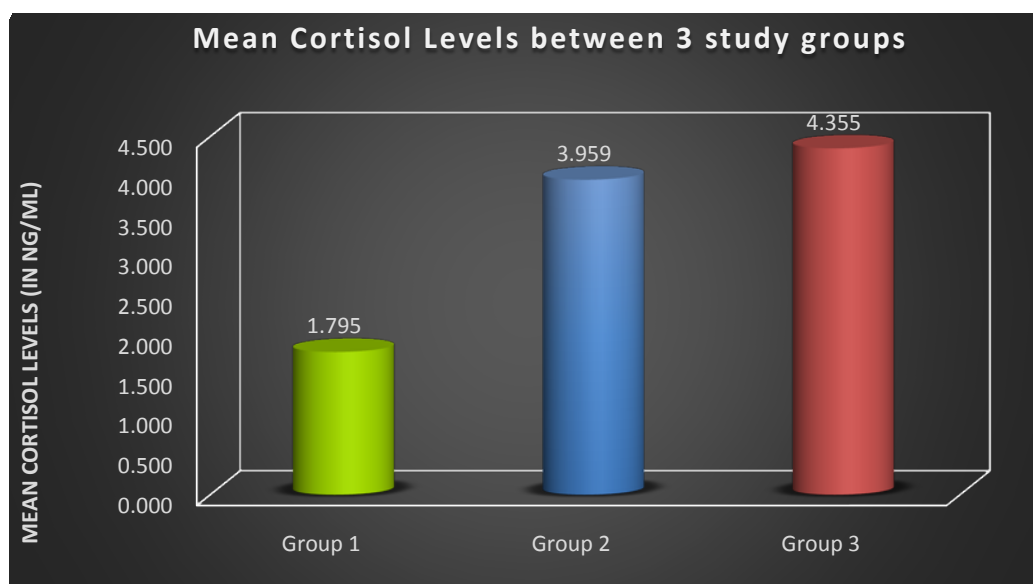
Comparison of mean Cortisol Levels (ng/ml) between three study groups using One-way ANOVA Test.

TABLE: 2

Groups	N	Mean	SD	Min	Max	P-value
Group 1	30	1.795	0.313	1.02	2.27	0.001*
Group 2	30	3.959	0.528	1.31	4.34	0.001*
Group 3	30	0.322	0.322	2.99	4.65	0.001*

* Statistically Significant.

Graph: 3



Multiple comparison of mean difference in Cortisol Levels (ng/ml) b/w three study groups using Turkey's post hoc Analysis.

TABLE: 3

Groups (I)	Group (J)	Mean diff (I-J)	95% CI for the Diff.	95% CI for the Diff.	P-value
			lower	upper	
Group 1					
	Group 2	-2.164	-2.410	-1.917	<0.001*
	Group 3	-2.559	-2.806	-2.313	<0.001*
Group 2	Group 3	-0.396	-0.642	-0.149	<0.001*

* Statistically Significant.

Multiple comparison of mean difference in anxiety sum scores b/w three study groups using Tukey's post hoc Analysis.

TABLE: 4

Groups (I)	Group (J)	Mean diff (I-J)	95% CI for the Diff.	95% CI for the Diff.	P-value
			lower	upper	
Group 1					
	Group 2	-1.33	-6.58	3.92	0.82
	Group 3	-8.23	-13.48	-2.98	0.001*
Group 2	Group 3	-6.90	-12.15	-1.65	0.007*

* Statistically Significant.

Pearson correlation test to assess the relationship b/w Anxiety sum scores & Cortisol levels in each study group.

TABLE: 5

Variables	Values	Group 1	Group 2	Group 3
Anx. Vs Cortisol	R	0.06	0.42	0.65
Anx. Vs Cortisol	P-Value	0.76	0.02*	<0.001*

The correlation coefficients are denoted by 'r'

Correlation coefficient range.

0.0 –No correlation.

0.01 - 0.20 - Very Weak Correlation.

0.21 - 0.40 –Weak Correlation.

0.41 - 0.60 - Moderate Correlation.

0.61 - 0.80 - Strong Correlation.

0.81 - 1.00 - Very Strong Correlation.

* Statistically Significant.

Simple linear regression analysis to predict the Cortisol levels by Anxiety scores in each group.

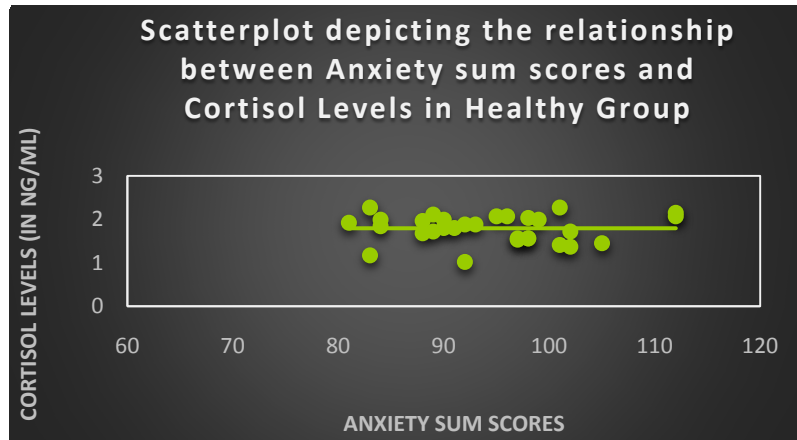
TABLE: 6

Groups	Variables	Co-efficient		T	P-value	R ²
		B	Std Error			
Group1	Constant	1.783	0.696	1.562	0.16	0.04
	Anxiety	0.000	0.007	0.017	0.99	
Group 2	Constant	2.377	1.358	1.751	0.09	0.16
	Anxiety	0.017	0.014	1.168	0.25	
Group 3	Constant	2.040	0.421	4.849	<0.001*	0.52
	Anxiety	0.023	0.004	5.529	<0.001*	

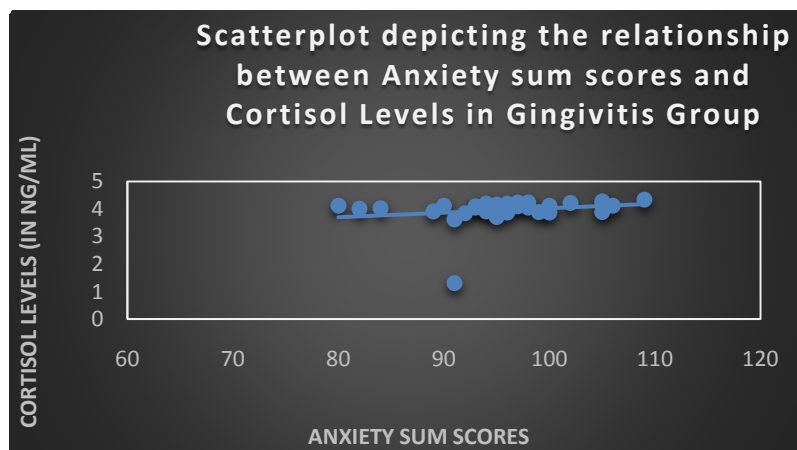
* Statistically Significant.

Scatter plot depicting the relationship between anxiety sum core and Cortisol level in healthy, gingivitis and chronic periodontitis.

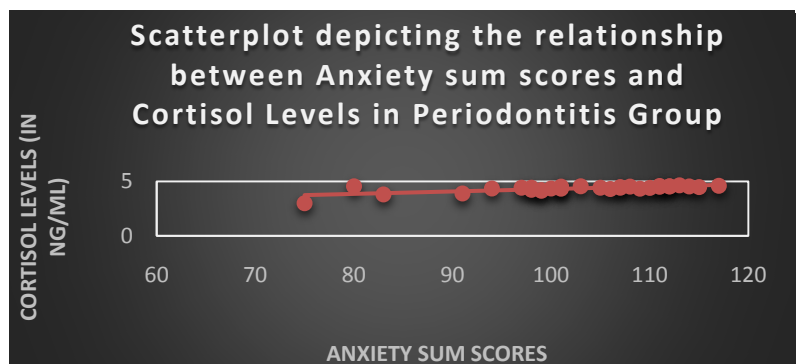
Graph: 4a, 4b, 4c



Graph: 4a



Graph: 4b



Graph: 4c

DISCUSSION

Everybody face stress in our day to day life, depending upon the duration of stress being exposed to an individual, there occurs a change in their neural and endocrine mechanism .The psychological stress can make an individual unable to cope up with his day to day activity, stress has an ability to cause immune-suppression and paves a pathway to periodontitis, more constant and unbearable stress can also lead to death.^{1,4}

Stress is one of the risk factors for periodontitis ⁶ apart from bacterial invasion which leads to periodontal degradation. Psychological depression and anxiety also play an additive role in periodontal destruction.^{1,2}

Cortisol is a corticosteroid which is released in stressful condition. During stressful condition, the fight, flight, flight response is being activated by the brain. So from the pituitary gland the information is mediated through a hypothalamus- pituitary-adrenal axis, which further activates the adrenal cortex and there is a release of cortisol, which circulates in the body, this cortisol level can be estimated from samples of blood, urine, saliva and gingival crevicular fluid. Patients under depression and Addison's disease have increased secretion of cortisol, stress can also affect pro-inflammatory cytokines, where the production of more amount of interleukin 1 and 6 and reduced level of IgA are noted.^{2,3,4,8}

The concentration of cortisol levels follows a circadian pattern, its level is high in morning and drops during the evening, the psychological mentality can be validated by various questionnaire scale, which is helpful to predict, the correlation between stress and its association with disease.³¹

So in case of periodontitis during the stressful condition, there is a release of inflammatory mediators, and the immune system of the body acts as a double-edged sword and leads to further destruction of periodontal structure.⁸

Apart from the immune response of the body due to stress, negligence of oral hygiene measures, improper diet, and development of habits such as smoking and alcohol during stressful conditions will further worsen the oral environment and make the oral cavity easily prone to periodontal pathogenic bacteria.² Carlos m et al conducted a study and concluded that there was an increase in *Prophyromonas gingivalis* count in individuals with increased serum cortisol level.²⁸

Many studies have stated that there is a strong correlation between stress and periodontitis, so the aims and objectives of this present study are to:

- Evaluate the salivary cortisol level in periodontally healthy, gingivitis and chronic periodontitis who are all systemically healthy.
- To investigate and compare the presence of salivary cortisol level among the three groups.
- To assess the correlation of salivary cortisol level and anxiety sum among three groups.

90 participants were selected for the study, all clinical parameters such as plaque index, gingival index, probing depth and clinical attachment level were recorded for categorizing individuals among the 3 groups, the healthy group, the gingivitis group, and the chronic periodontitis group who are all systemically healthy. The participants were asked to report to department of periodontics in K.S.R institute of dental science and research, with fasting and without brushing in morning during clinical hours, around 8:30

am to 9:30 am and the unstimulated saliva samples were collected in a sterile container, and centrifuged at 15 mins for 2500 rpm and stored in a deep freezer at -18 degree Celsius until the day of analysis, and the samples were analyzed using salivary cortisol ELISA kit and psychometric analysis was done with STAI inventory questionnaire scale.

The results of this present study showed that there is a significant increase in salivary cortisol levels in chronic periodontitis patients (group-3) when compared to patients with gingivitis (group-2) and periodontally healthy (group-1).

There was a moderate level of salivary cortisol increase in the gingivitis group when compared to chronic periodontitis and healthy group. The healthy group had lesser salivary cortisol levels when compared between the other 2 groups.

Similarly, the mean values of the STAI were higher in the periodontitis group and it correlates well with the higher cortisol value of the same group when compared to the other 2 groups.

The mean anxiety score among the 3 groups, 1, 2 and 3 are 94.33, 95.67 and 102.57 and the mean salivary cortisol levels of the 3 groups are 1.795, 3.959 and 4.355. On further comparing the mean of salivary cortisol level and anxiety sum of the groups, 1, 2 and 3 are 0.06, 0.42 and 0.65.

Thus in our study, the salivary cortisol level and anxiety level were highly correlated and associated with the chronic periodontitis group. The result of this study was similar with other studies as follows,

Cakmal et al (2016)³¹ conducted a study between aggressive periodontitis, chronic periodontitis, and healthy subjects and concluded that there is an increase in the level of

salivary cortisol in aggressive periodontitis and chronic periodontitis group when compared to healthy subjects.³¹

Refulio et al (2013)²⁹ conducted a study among 70 individuals of healthy and chronic periodontitis groups by analyzing saliva samples. He concluded that there is an increased salivary Cortisol level in the periodontitis group when compared to healthy individuals.

Mahendra et al (2011)²⁶ conducted a study to evaluate the salivary Cortisol level among 110 individuals. He categorized the individuals upon the values of probing pocket depth and then collected the saliva samples. Occupation stress questionnaire was answered and the results stated, that individuals with high occupational stress had more probing depth and prone for periodontitis.

Rosania et al (2009)²³ conducted a study between psychological markers and stress marker between 45 samples and concluded that patients with increased mobility, probing depth and teeth loss have increased salivary Cortisol level.

Mannem et al (2019)³⁷ et al 2019 conducted a study to assess the relationship between stress and salivary Cortisol level in 111 individuals, morning saliva samples were collected, all clinical parameter were measured, a LIPPS scale was used as a psychoanalytical questionnaire. The individual with higher mean Cortisol level and anxiety was associated with periodontitis.³⁵

Mudrika et al (2019)³⁸ et al 2019 conducted a study to find the relationship between stress and periodontitis. 35 individuals were selected for the study, all clinical parameters were measured and Becks psychoanalytical scale was answered by all the

samples. The individuals with increased probing depth had a positive correlation with the psychoanalytical scale.

The limitations of this study are:

- Serum samples can also be taken along with saliva samples for better results.
- 3 intervals of saliva samples would have been collected for more accuracy of results.
- A Comparison of the periodontitis group with age can be helpful to determine which age groups are more affected by stress.

SUMMARY AND CONCLUSION

This cross-section study was done to evaluate and assess the presence of Cortisol level in saliva and its relationship with periodontal disease progression.

The study population is 90 individuals and they were separated into three groups, healthy (group-1), gingivitis (group-2) and chronic periodontitis (group-3) who are all systemically healthy. All these patients were selected from the outpatient ward of K.S.R. institute of dental science and research. The morning saliva samples were collected from the individuals for analysis, using ELISA kit and the anxiety state of the individuals was measured using STAI scale.

These are the following conclusion of this study:

- The salivary cortisol levels were elevated in group-3, the chronic periodontitis group when compared to the other 2 groups (healthy and gingivitis).
- The salivary cortisol level of gingivitis group was moderately higher when compared to the healthy group.
- The healthy group had a weak correlation of salivary cortisol values.
- The STAI scale was measured to analyze the anxiety level of all the individuals in all three groups, where the anxiety sum was higher to periodontitis group when compared to the healthy and gingivitis group.
- The mean value of salivary cortisol level and the anxiety sum had a higher correlation when compared to the other two groups.

Based on the results obtained from the study, it has been concluded that salivary cortisol level was elevated and moderately raised in chronic periodontitis individuals and gingivitis individuals with a positive correlation to the STAI inventory anxiety scale.

Many studies have been one to determine, the role of salivary cortisol in periodontics. Similarly this study also showed a positive correlation to the other older studies, thus Cortisol can be used as a stress biomarker in periodontics.

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ANNEXURE 1

INFORMATION SHEET

We are conducting a study on-“**STRESS CORTISOL AND PERIODONTAL STATUS-A CROSS SECTIONAL STUDY**”

The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared. Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled. The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date

ANNEXURE 2

INFORMED CONSENT FORM

**STRESS, CORTISOL AND PERIODONTAL STATUS-A CROSS SECTIONAL
STUDY.**

K.S.R INSTITUTE OF DENTAL SCIENCE AND RESEARCH.

DEPARTMENT OF PERIODONTICS.

Ihereby declare that I have read this consent and clearly understood the procedures, possible discomforts, as well as possible benefits of the study. I permit the above-mentioned institution/individual/organization/hospital to disclose my records to the individual/organization listed above.

SIGNATURE: DATE:.....

I have explained the procedure involved in the study and answered all the questions asked by the patient.

SIGNATURE: DATE:

ஒப்புக்கைவாக்குமுலம்

ஆகியநான்மேற்கூறியஆராய்ச்சிபடிப்பின்வழிமுறைகளைத்தெளி

வாகப்புரிந்துகொண்டேன்

.மேலும்நான்இந்தஆராய்ச்சிபடிப்புக்கானவழிமுறைகளைமேற்கொ

ள்வதற்கும்அதன்பரிசோதனைமுடிவுகளைதெரிந்துகொள்ளவும்மு

ழுமையாகஅனுமதிக்கிறேன்.

நோயாளியின்கையொப்பம்தேதி -----

நான்மேற்கூறியஆராய்ச்சிபடிப்பின்வழிமுறைகள்மற்றும்அதுகுறித்

தநோயாளியின்சந்தேகங்களையும்தெளிவாகவிளக்கியுள்ளேன் .-----

மருத்துவரின்கையொப்பம்தேதி -----

ANNEXURE 3

STRESS, CORTISOL AND PERIODONTAL STATUS-A CROSSECTIONAL

STUDY

PROFORMA

S.No :

O.P.no:

Date:

Name:

Age/Gender:

Occupation:

Address:

Contact No:

Chief complaints:

Past Medical history:

Past Dental history:

Laboratory investigations:

PERIODONTAL EXAMINATION

PLAQUE INDEX – MODIFIED BY LOE (1967)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

PI score=

GINGIVAL INDEX – MODIFIED BY LOE(1967)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

GI score:

PROBING POCKET DEPTH (in mm)

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Mean-

State-Trait Anxiety Inventory for Adults

Self-Evaluation Questionnaire STAI Form Y-1 and Form Y-2

Developed by Charles D. Spielberger

in collaboration with R.L. Gorsuch, R. Lushene, P.R. Vagg, and G.A. Jacobs

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STAIP-AD Test Form Y
www.mindgarden.com

SELF-EVALUATION QUESTIONNAIRE STAI Form Y-1

Please provide the following information:

Name _____ Date _____ S _____

Age _____ Gender (Circle) **M** **F** T _____

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

VERY MUCH SO
MODERATELY SO
SOMEWHAT
NOT AT ALL

- | | | | | |
|--|---|---|---|---|
| 1. I feel calm..... | 1 | 2 | 3 | 4 |
| 2. I feel secure | 1 | 2 | 3 | 4 |
| 3. I am tense | 1 | 2 | 3 | 4 |
| 4. I feel strained | 1 | 2 | 3 | 4 |
| 5. I feel at ease | 1 | 2 | 3 | 4 |
| 6. I feel upset | 1 | 2 | 3 | 4 |
| 7. I am presently worrying over possible misfortunes | 1 | 2 | 3 | 4 |
| 8. I feel satisfied | 1 | 2 | 3 | 4 |
| 9. I feel frightened | 1 | 2 | 3 | 4 |
| 10. I feel comfortable | 1 | 2 | 3 | 4 |
| 11. I feel self-confident | 1 | 2 | 3 | 4 |
| 12. I feel nervous | 1 | 2 | 3 | 4 |
| 13. I am jittery | 1 | 2 | 3 | 4 |
| 14. I feel indecisive..... | 1 | 2 | 3 | 4 |
| 15. I am relaxed | 1 | 2 | 3 | 4 |
| 16. I feel content | 1 | 2 | 3 | 4 |
| 17. I am worried | 1 | 2 | 3 | 4 |
| 18. I feel confused..... | 1 | 2 | 3 | 4 |
| 19. I feel steady..... | 1 | 2 | 3 | 4 |
| 20. I feel pleasant..... | 1 | 2 | 3 | 4 |

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STAIP-AD Test Form Y
www.mindgarden.com

SELF-EVALUATION QUESTIONNAIRE

STAI Form Y-2

Name _____ Date _____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

ALMOST NEVER
SOMETIMES
OFTEN
ALMOST ALWAYS

- | | | | | |
|--|---|---|---|---|
| 21. I feel pleasant..... | 1 | 2 | 3 | 4 |
| 22. I feel nervous and restless | 1 | 2 | 3 | 4 |
| 23. I feel satisfied with myself..... | 1 | 2 | 3 | 4 |
| 24. I wish I could be as happy as others seem to be | 1 | 2 | 3 | 4 |
| 25. I feel like a failure | 1 | 2 | 3 | 4 |
| 26. I feel rested | 1 | 2 | 3 | 4 |
| 27. I am "calm, cool, and collected"..... | 1 | 2 | 3 | 4 |
| 28. I feel that difficulties are piling up so that I cannot overcome them..... | 1 | 2 | 3 | 4 |
| 29. I worry too much over something that really doesn't matter..... | 1 | 2 | 3 | 4 |
| 30. I am happy | 1 | 2 | 3 | 4 |
| 31. I have disturbing thoughts | 1 | 2 | 3 | 4 |
| 32. I lack self-confidence..... | 1 | 2 | 3 | 4 |
| 33. I feel secure | 1 | 2 | 3 | 4 |
| 34. I make decisions easily | 1 | 2 | 3 | 4 |
| 35. I feel inadequate..... | 1 | 2 | 3 | 4 |
| 36. I am content | 1 | 2 | 3 | 4 |
| 37. Some unimportant thought runs through my mind and bothers me | 1 | 2 | 3 | 4 |
| 38. I take disappointments so keenly that I can't put them out of my mind..... | 1 | 2 | 3 | 4 |
| 39. I am a steady person..... | 1 | 2 | 3 | 4 |
| 40. I get in a state of tension or turmoil as I think over my recent concerns
and interests | 1 | 2 | 3 | 4 |

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STAI-AD Test Form Y
www.mindgarden.com

State-Trait Anxiety Inventory for Adults Scoring Key (Form Y-1, Y-2)

Developed by Charles D. Spielberger in collaboration with R.L. Gorsuch, R. Lushene, P.R. Vagg, and G.A. Jacobs

To use this stencil, fold this sheet in half and line up with the appropriate test side, either Form Y-1 or Form Y-2. Simply total the scoring **weights** shown on the stencil for each response category. For example, for question # 1, if the respondent marked 3, then the **weight** would be 2. Refer to the manual for appropriate normative data.

Form Y-1					Form Y-2				
	NOT AT ALL	SOMEWHAT	VERY MUCH SO	MODERATELY SO		ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
1.	4	3	2	1	21.	4	3	2	1
2.	4	3	2	1	22.	1	2	3	4
3.	1	2	3	4	23.	4	3	2	1
4.	1	2	3	4	24.	1	2	3	4
5.	4	3	2	1	25.	1	2	3	4
6.	1	2	3	4	26.	4	3	2	1
7.	1	2	3	4	27.	4	3	2	1
8.	4	3	2	1	28.	1	2	3	4
9.	1	2	3	4	29.	1	2	3	4
10.	4	3	2	1	30.	4	3	2	1
11.	4	3	2	1	31.	1	2	3	4
12.	1	2	3	4	32.	1	2	3	4
13.	1	2	3	4	33.	4	3	2	1
14.	1	2	3	4	34.	4	3	2	1
15.	4	3	2	1	35.	1	2	3	4
16.	4	3	2	1	36.	4	3	2	1
17.	1	2	3	4	37.	1	2	3	4
18.	1	2	3	4	38.	1	2	3	4
19.	4	3	2	1	39.	4	3	2	1
20.	4	3	2	1	40.	1	2	3	4

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STAIP-AD Scoring Key
www.mindgarden.com

RESEARCH METHODOLOGY AND BIOSTATISTICS



The Tamil Nadu Dr.M.G.R. Medical University
69, Anna Salai, Guindy, Chennai - 600 032.



DEPARTMENT OF EPIDEMIOLOGY
CREDIT POINTS : 30

This certificate is awarded to Dr./Mr./Ms. **LAKSHANA S**
for participating as a Delegate in the three days Workshop on 'Research
Methodology and Biostatistics : How to do a Good Dissertation & Publish?'
from 18 - 12 - 2019 to 20 - 12 - 2019.

Dr.G.SRINIVAS
PROFESSOR & HEAD
DEPARTMENT OF EPIDEMIOLOGY

Dr.PARAMESWARI SRIJAYANTH
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 - P.S. Raja, B.A.,**
(Person)

Date : 19.12.2016

Ref.: 162/KSRIDSRE/EC/2016

To

Dr.S.Lakshana,
Postgraduate Student,
Dept. of Periodontics,
KSR Institute of Dental Science & Research,

Your dissertational study titled "STRESS CORTISOL AND PERIODONTAL STATUS: A CROSS SECTIONAL STUDY" presented before the ethical committee on 16th Dec. 2016 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

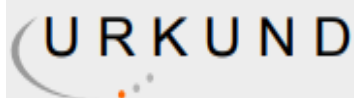
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