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Salivary Cortisol and Total Count of Subgingival Aerobic/ Anaerobic Bacteria among Chronic Periodontitis Iraqi Armored Forces (Comparative Study)

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ABSTRACT

Background: Chronic periodontitis is a critical widespread health problem among population in both developing and industrialized countries with several etiological and associated risk factors. Many aerobic and anaerobic bacteria like aggregatibacter actinomycotemcomitans (a gram negative coccobacillus) and Porphyromonas Gingivalis act synergistically associated with this type of disease. Hypothalamicpituitary-adrenal axis release cortisol hormone which exerted in saliva ,and the drop of salivary melatonin hormone levels which act as antioxidant, anti-inflammatory and immunomodulatory agent, might tip the host–bacteria interaction in a favour of bacteria causing increase in off attachment loss .also stress caused by elevated cortisol hormone may affect how well people look after their oral hygiene and daily plaque removal which leads to development of periodontal disease especially chronic periodontitis .

Aim of the study: this study aims to determine the difference in the level of salivary cortisol,total count of aerobic and anaerobic bacteria and anxiety among chronic periodontitis group and periodontitis free group among Iraqi armored forces member and to evaluate the impact of salivary cortisol, total count aerobic and anaerobic bacteria and anxiety on the development of chronic periodontitis .

Material and methods: One hundred sixty subjects included in this study divided into two groups:

Control group: Eighty subjects with healthy chronic periodontitis free, this group exposed to the basic periodontal examination.

Study group: Eighty subject with chronic periodontitis.

Salivary cortisol level measured and periodontal plaque serially diluted and cultured under aerobic and aerobic conditions for each group.

Anxiety estimated for each group by anxiety questionnaire (GAD-7).

Result: There was significant impact of salivary cortisol over the study, total count of bacteria, and anxiety.

Key words: Chronic periodontitis, Salivary cortisol, Anxiety, Bacterial total count

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INTRODUCTION

Periodontitis is the response to periodontal inflammation that leads to the tooth-supporting tissues destruction. It is one of tooth loss most prevalent causes [1]. Chronic periodontitis disease (CP) is multifactorial, with a complex interaction between microbial infection and host response in which the consideration of dental biofilms microbiology as the main etiological agents for inflammation initiation [2]. Asaccharolytic bacteria gram-negative anaerobe (*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*) are in significant correlation with periodontitis. *P. gingivalis* has been approved in correlation with periodontal destruction and has an multiple of virulent factors that affect stimulation responses of the host. T. forsythia became a recognized periodonto pathogen due to its continuous detection from periodontitis sites and its correlation with the formation of deep size pocket [3]. In periodontitis subgingival sites, *T. denticola* is additionally frequently presented and after appropriate treatment, their number is decreased [4]. *Fusobacterium nucleatum, Selenomonas, Prevotella intermedia, Prevotella nigrescens, Eikenella corrodens, Eubacteria, Parvimonas micra* and Campylobacter rectus are included as related with periodontitis [5]. Although periopathogenic bacteria presence is essential to initiate the process but their mere presence is not sufficient [6]. The risk of psychological stress as an absolute risk factor is still not known in the periodontal disease development [7,8]. However host resistance to periopathogenic bacteria can be affected by the association between the periodontitis development with conditions. Evidence suggests that chronic stress, depression, and anxiety may affect the progression of chronic periodontitis (CP) in a

positive way [9,10]. A glucocorticoid hormone (Cortisol) secreted by adrenal glands, can effect on the physiological structures of body variously. Physiological or neurological type of stress can increase the release of adrenocorticotropic hormone immediately and significantly from the anterior pituitary gland, lead to increase in cortisol secretion from the adrenal cortex in a few minutes. Elevated cortisol remains persistent in the chronic stress case [11]. Salivary cortisol in moderate and severe CP patient had significantly higher than mild CP patients and those patients have an increased risk for CP due high salivary cortisol level, and depression [2]. Correlation is significant between hypercortisolemia, work tension, economic problems, clinical stress syndrome and patients with CP [12].

MATERIAL AND METHODS

Study population

Conduction of this study were in Al–Muthana military hospital, approved by the ethical committee of Baghdad university, college of dentistry following the roles of Tokyo and Helsinki for human .all sample were collected between January2020 till February2021. All individual(Iraqi armored forces members) aged between 20 -45 years were voluntary participated to the study after consent form signature and submitted to questionnaire including their name, age, gender, medical history, chronic disease, and smoking following by Basic periodontal examination. Exclusion criteria involved chronic diseases, smoking; medication that interferes with measurement salivary cortisol hormone, individual ate before one hour from sample collection and female individual were excluded.

Study design

One hundred sixty all male individual divided into two groups, 80 participant into study group with chronic periodontitis and (80) participant free of chronic periodontitis according to basic periodontal examination (BPE) were chosen to be the control group. Questionnaire about the general health and oral health habits of the selected Iraqi Armored Forces Member (IAFM) was prepared to ensure that they were free from any systematic diseases and didn't take any medications for at least the last fourteen days.

Periodontal pocket plaque collection and preparation for culturing

After periodontal pocket examination , pocket plaque collection process take place by inserting sterile paper point into the full depth of periodontal pocket , then we collect the plaque then putting it in plain test tube filled with PBS and glass peads ,with the vibrating motion executed by vortex, glass peads will shed a pressure on the paper point to extract the plaque and to be mixed with PBS .Serial dilution done by taking 0.1 ml PBS plaque mixture added to 9.9ml of PBS to have 10-3, 10-5 dilutions which now ready to be cultured in aerobic and anaerobic conditions.

Saliva sampling

Saliva collection tubes should be prepared and numbered before saliva collection. In the morning between 8-10 am, unstimulated saliva was collected. The involved IAFM should not eat or drink one hour before the collection. After that, instructions had been given to the IAFM to take care about their teeth and their oral hygiene. Resting saliva was centrifuged for 3000 rpm for 20 minutes and the clear supernatant was collected and stored in freezer at -20°C until the determinations of salivary cortisol was done [13].

Biochemical assessment

Salivary cortisol kit based on cortisol-specific biotinylated antibody and a ruthenium complex labelled cortisol derivative. Depending on the concentration of the analyte in the sample and the formation of the respective immune complex, the labelled antibody binding site is occupied in part with the sample analyte and in part with the ruthenylated hapten. After addition of streptavidin-coated micro particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Statistical analysis

Statistical analysis was done by using statistical package for social sciences (SPSS). In testing normality among groups for both parameters, Shapiro-Wilk test used. Parametric details were demonstrated as mean ± SD. Paired T test were used to compare between study group and control group. Microsoft excel program was used to estimate the correlation between salivary cortisol hormone, anxiety and bacterial total count.

RESULTS

Findings in Table 1 illustrated that salivary cortisol, found to be higher in the chronic periodontitis (study group) with (Mean 8.81, SD 0.98) than chronic periodontitis free (control group) (Mean 7.63, SD 1.43), results found to be significant difference with P-value<0.05. findings in Table 2 reveals that anxiety score found to be higher in study group with (Mean 8.81, SD 3.69) than control group (Mean 11.9 ,SD 3.96), results found to be significant difference with P-value<0.05.Findings in Table 3 illustrated that bacterial total count in same manner of salivary cortisol and anxiety , found to be higher in study group (Mean 5.65, SD 2.526) than control group (Mean 4.85, SD 2.51) , results found to be significant difference with P-value<0.05 .Correlation between salivary cortisol and anxiety found to be positive direct relation as in Table

4.The impact salivary cortisol over the total count of bacteria was significant and found to be positive relation as in Table 5 and Table 6.

Table 1: Descriptive of salivary cortisol hormone between control and study.

Variables	Control group N=80		Study group N=80		Statistical analysis			
cortisol hormone	Mean	SD	Mean	SD	t-test	df	P-value	Sig
	7.63	1.43	8.18	0.98	2.079	79	0.008	S
*P<0.05 Significant								

Table 2: Descriptive of Anxiety records between control and study.

Variables	Control group N=80		Study group N=80		Statistical analysis			
Anxiety	Mean	SD	Mean	SD	t-test	df	P-value	Sig
	8.81	3.69	11.9	3.96	4.901	79	0.0004	S
*P<0.05 significant								

Table 3: Descriptive of total count between control and study.

Variables	Control N=80		Study N=80		Statistical analysis			
Total count	Mean	SD	Mean	SD	t-test	df	P-value	Sig
5th dilution	1.575	0.143	2.037	0.157	2.43	79	0.017	S
*P<0.05 Significant								

Table 4: Descriptive Comparison between anxieties with cortisol hormone.

Variables	Control			Study		
	t-test	P-value	Sig	t-test	P-value	Sig
	10.56	0.008	S	7.39	0.009	S
*P<0.05 Significant						

Table 5: Simple linear regression analysis between the anxiety and cortisol level.

Variable	Anxiety					
cortisol level	No.	R	R-Square	β	t-test	P-Value
Study	80	0.415	0.172	1.143	4.032	0.003
Control	80	0.788	0.06	0.294	0.691	0.492

Table 6: Simple linear regression analysis between the cortisol and total count.

Variable		Cortisol					
Total count	Dilution	No.	R	R-Square	β	t-test	P-Value
Control	5th dilution	80	0.056	0.043	0.032	0.494	0.622
Study	5th dilution	80	0.201	0.04	0.127	1.83	0.004

DISCUSSION

Regarding the salivary cortisol hormone and genera; anxiety scale (GAD-7) statistical analysis , difference was significant between control group and study group with (P value <0.05) and direct positive relation between both of them according to the fact that the subjects of study group had chronic periodontitis , on the other hand , control group found to have a normal healthy periodontium. These results agree with many studies, Refulio et al. and Mannem et al. [2,12] which shows that elevation of salivary cortisol hormone in subjects having chronic periodontitis . Kaufman et al. [14] found that salivary cortisol levels that reflected by emotional stress, is a periodontal disease risk factor and behavioural changes can be the result of stress, which significantly could affect periodontium. Genco et al. [15] concluded salivary cortisol was used in emotional stress evaluation in periodontal disease. In individuals exhibiting severe periodontitis higher salivary cortisol levels were detected, a high level of emotion focused coping, and high financial strain, as compared to individuals with no or little periodontal disease, low emotion-focused coping, and low levels of financial strain. . Deinzer et al.[16] and Ishisaka et al. [17] suggested that psychosocial stress may influence the oral hygiene and this may alter the extent and severity of periodontitis. Hana et al. [18], Angela et al. [19] Takaia et al. [20]. They found that increases salivary cortisol in response to anxiety and stress, and that it is also presents an noninvasive, easy way of stress measuring.

Regarding bacterial total count, the difference was a significant between study group and control group due to the fact that control subjects had good oral hygiene and healthy periodontal tissues according to the selection criteria. These findings agree with result of Listgraten [21]. Plaques harvested microbial profiles of from healthy gingival sulci differ from those took from periodontitis or gingivitis lesions. Current study finding agreed with Hamdoon SM [22], study that found a positive relation between the bacteria spp. count and the pocket depth in CP patients. Boyanova et al [23] and Kores et al. [24] found a very high prevalence of a complex strict anaerobic Gram-negative rod in sub gingival plaque samples of deep pockets and demonstrated that *Prevotella intermedia* and *A. actinomycotemcomitans* were highest in patients with pocket depth >3 mm respectively which is similar to current study findings. Boyce et al. [25] study reveals that salivary cortisol have direct positive relation with bacterial total count, higher cortisol level might suppress the oral immunity and induce the proliferation of the bacteria. An increase in salivary cortisol may affect the microbial colonization processes such as adhesion and cohesion [26]. Atrophic change of the major salivary glands caused by corticosteroids may lead to decrease in the quantity (volume) and the composition of the saliva; meanwhile, decreasing the salivary secretion will reduce the clearance of bacteria Jain et al. [27].

CONCLUSION

Salivary cortisol hormone level elevated in chronic periodontist patients which lead to poor prognosis treatment and results and can be used to monitor periodontal health. SCl has direct positive relation with anxiety and total bacterial count.

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