Effect of the examination stress on periodontal health status and salivary IL-1β among Iraqi dental students

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ABSTRACT

Background: Periodontal diseases (PD) are common chronic inflammatory diseases caused by pathogenic microorganisms colonizing the gingival area and inducing local and systemic elevations of pro-inflammatory cytokines resulting in tissue destruction by a destructive inflammatory process. Stress was considered as one of the important risk factors that cause many inflammatory diseases including PD. The purpose of this study wasto determines and compares clinical periodontal parameters (PLI, GI and BOP), stress level and salivary IL-1β level among dental students before, during and after mid-year exam, also to find the correlation among stress, IL-1β and clinical periodontal parameters.

Materials and methods: The sample was consisted of 24 dental students; 12 male and 12 female aged (21-23) years, theywere examined in this follow up study at three main periods; first period at least one month before mid-year exam (Period I), second period during mid-year exam (Period II) and third period at least one month after mid-year exam (Period III). DASS-21 was used to measure stress level in all periods. Saliva samples were collected to determine the salivary IL-1β level by ELISA. Clinical periodontal parameters were recorded at four sites per tooth.

Results: The means of all clinical periodontal parameters were higher in the period II than in the periods I and III with highly significant differencesat ($P \leq 0.01$). As well as, the means concentrations of salivary IL-1β were higher in the period II than in the periods I and III with highly significant differencesat ($P \leq 0.01$). Also, by using Pearson's Correlation Coefficient, stress shows highly significant strong correlation with IL-1β and clinical periodontal parametersat ($P \leq 0.01$).

Conclusions: The results of this study provided strong evidence of association between examination stress and PD, where dental students during mid-year exam have higher levels of stress, clinical periodontal parameters and salivary IL-1β as compared with before and after mid-year exam periods.

Key words: Examination stress, IL-1β, saliva, DASS-21, periodontal health. (J Bagh Coll Dentistry 2013; 25(4):72-78).

الخلاصة

الخلفية:أمراض ما حول الأسنان هي أمراض التهابيه مزمنة تسببها كائنات حية دقيقه ممرضه تستعمر منطقه اللثةوتحفز على ارتفاعات موضعية وعامة في المدورات الخلوية المؤدية للالتهابات مما يؤدي إلى تدمير الأنسجة بواسطة عملية الالتهاب المدمر. الإجهاد يعتبر كواحد من العوامل المهمة الخطرة التي تسبب العديد من الأمراض الالتهابية بما في ذلك أمراض ما حول الأسنان. الغرض من هذه الدراسة لتحديد ومقارنة مؤشرات ما حول الأسنان، مستوى الأجهاد ومستوى البين ابيضاضي1 بيتا اللعابي بين طلاب طب الأسنان قبل، خلال وبعد فترة امتحان نصف السنة. وبالإضافة إلى ذلك، لإيجاد العلاقة بين الإجهاد، البين ابيضاضي1 بيتا اللعابي والمؤشرات السريرية لما حول الأسنان.

المواد والطرق: العينة تكونت من 24 طالبا لطب الأسنان ; 12 ذكرا و12أنثى تتراوح أعمارهم بين (21-23) عاما، الذين تم فحصهم في هذه الدراسة المتتابعة في ثلاث فترات رئيسية:الفترة الأولى بشهر واحد على الأقل قبل امتحان نصف السنة (الفترة I)، الفترة الثانية خلال امتحان نصف السنة (الفترة II)، الفترة الثالثة بشهر واحد على الأقل بعد امتحان نصف السنة (الفترة III). استخدمDASS-21 لقياس مستوى الإجهاد في كل المجموعات.تم جمع عينات من اللعاب لتحديد مستوى البين ابيضاضي1 بيتا اللعابي بواسطةELISA.

النتائج:المتوسطات الحسابية لجميع مؤشرات ما حول الأسنان السريرية كانت أعلى في الفترة الثانية مما كانت عليه في الفترات الأولى والثالثة مع وجود اختلافات معنوية عالية عند ($P \leq 0.01$).وكذلك، الاوساط الحسابي لتركيز البين ابيضاضي1 بيتا اللعابي كانت أعلى في الفترة الثانية مما كانت عليه في الفترات الأولى والثالثة مع وجود اختلافات معنوية عالية عند ($P \leq 0.01$).كذلك، باستخدام معامل ارتباط بيرسون،يشهر الإجهاد وجود علاقة معنوية قوية مع (البين ابيضاضي1 بيتا اللعابي) والمؤشرات لما حول الأسنان عند.($P \leq 0.01$)

الاستنتاج:قدمت نتائج هذه الدراسة دليلا قويا على الارتباط بين إجهاد الامتحان وأمراض ما حول الأسنان، حيث أن طلاب طب الأسنان خلال امتحان نصف السنة امتلكوا مستويات أعلى للإجهاد ومؤشرات ما حول الأسنان والبين ابيضاضي1 بيتا اللعابي بالمقارنة مع فترات قبل وبعد امتحان نصف السنة.

الكلمات الرئيسية:اجهاد الامتحان، IL-1β، اللعاب، DASS-21، وصحة ما حول الاسنان.

INTRODUCTION

Periodontal diseases are multifactorial infection characterized by destructive inflammatory process affecting tooth-supporting tissues caused by pathogenic microorganisms, which induce elevations of pro-inflammatory cytokines resulting in tissue destruction. Evolution of PD is influenced by many local or systemic risk factors [(1)]. Dental plaque, which harbours specific periodontal pathogens, is a primary etiologic factor. Where host tissue damage in PD is mainly due to the action of oral microbes and associated host immune-inflammatory responses [(2)]. In addition, several risks and susceptibilities have been associated with PD, like systemic diseases, smoking and psychological stress.

Several clinical studies have investigated the possible relationship between psychological stress and PD and have suggested that stress may play a role in development of PD [(3,4)]. Stress is defined as the reactions of the body to forces of a deleterious nature, infections and various abnormal states that tend to disturb its normal physiological equilibrium [(5)]. It's nevertheless a confirmed and important factor in the etiology and maintenance of many inflammatory diseases, including PD [(6)]. It's said to influence the host defenses, exerting an immunosuppressive effect, increasing one's vulnerability to disease. Cytokines and other humoral mediators of inflammation are potent activators of the central stress response, and the glucocorticoids released via this mechanism might regulate the recruitment of immune cells

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into inflamed tissues, in order to cope with the psychological stress and depression [7]. When the inflammatory action is sufficiently long and profound, the systemic manifestations of the disease may become evident, as could happen with PD.Many physiopathological processes are involved in periodontal destruction in terms of the inflammatory and immune host response, especially proinflammatory cytokines [8,9].

IL-1β is a highly pro-inflammatory cytokine strongly associated with periodontal breakdown [10]. Also, IL-1, produced following exposure to immunological and psychological challenges, plays an important role in the neuroendocrine and neurobehavioral stress responses [11].Saliva is a mirror to the general health condition that reflects various systemic changes in the body [12].Where the composition of saliva immediately reflects the sympathetic and parasympathetic nervous systems, hypothalamic-pituitary-adrenal (HPA) axis and immune system response to stress [13]. In addition, salivary levels of various biochemical parameters have been measured in infectious diseases and psychiatric disorders [14].

MATERIALS AND METHODS

The original sample was consisted of 54 dental students, 23 male and 31 female aged (21-23) years were randomly recruited in this follow up study. They were fifth class students from the collage dentistry in University of Baghdad and Al-Mustansiriyah; 11 males and 19 females were excluded from this study in the second and third periods because they were not fit for the criteria of study. Therefore, the final sample was 24 students; 12 males and 12 females continue to follow in this study at three main periods. The students enrolled voluntarily in the study after a well explanation of purpose of the investigation and consented to its protocol in period from November 2012 to March 2013. All students in this study were systemically healthy, cooperative, nonsmoker and not taking any antibiotics during the last three months [15].Any pregnant and in menstrual cycle women, student had history of chronic systemic diseases with known associations with PD (e.g. diabetes mellitus), student taking psychotropic medication (e.g. prednisone) and any student with retentive factor of dental plaque (e.g. orthodontic appliance) were excluded from this study.Clinical periodontal parameters were measured and stress questionnaire were recorded at three main periods:

1. Period I (at least 1 month before mid-year exam): Students were in the normal range of stress scale of DASS criteria and all students given motivation and instructions about brushing technique and the use of dental aids to reduce accumulation of dental plaque and gingival inflammation.Itused as a base line.
2. Period II (during mid-year exam): Students subjected to stress, again all students given motivation and instructions about brushing technique and the use of dental aids.
3. Period III (at least 1 month after mid-year exam): Students were in the normal range of stress scaleof DASS criteria.

DASS-21 was used to measure stress level in all periods. Students were answered about stress questionnaires during the three periods before the examination of clinical periodontal parameters; Respondents were asked to use 4-points of severity scales to rate the extent to which they have experienced each state over the past week. Scores of stress calculated by summing the scores for the relevant items. Then, the sum for stress scores evaluated by the severity-rating index [16].

Saliva sample collection and preparation

The students were instructed not eat or drink (except water) for at least 60 minutes before collection of the samples,acidic or high sugar foods can compromise assay performance by lowering saliva PH and influencing bacterial growth. To minimize the effect of these factors, the student was rinsed his/her mouth thoroughly with water then waited 2 minutes for water clearance then subject was sat in a relax position then the whole unstimulated mixed saliva was collected into the polyethylene tubeuntil 2ml was collected. Saliva was collected between 8-12 a.m.After sample collection, it was put in a cooling box within 30 minutes and freezing at (-70ºC) within 4 hours of collection until assayed, in order to prevent bacterial growth and minimize loss of IL-1β in the sample.Freezing saliva samples were precipitate mucins. On day of assay, thaw completely then centrifuge at 3000 rpm for 15 minutes. For determination of IL-1β in saliva, commercially available ELISA was used and performed as recommended in leaflet with kit (Salimatrics Company, USA).

RESULTS

The mean of PLI was elevated in period II in comparison with other periods, Table (1).For comparisons among periods, ANOVA test was used; the results showed that there were HS difference at P-value ≤ 0.01 among and within periods, Table (2). LSD was performed for multiple comparisons between each two periods; the results showed that there were HS differences at P-value ≤ 0.01, Table (3). Also; the mean of GI

was elevated in the period II in comparison with other periods (4).ANOVA test was showed that there were HS difference at P-value ≤ 0.01 among and within periods, Table (5). LSD test was showed that there were HS differences at P-value ≤ 0.01 between periods I and II; II and III, while there was NS difference at P-value ≥ 0.05 between periods I and III as shown in Table (6).The number and percentage of bleeding sites in the period II was higher than the periods I and III. For comparison among periods, chi-square test was showed that there was HS difference at P-value ≤ 0.01 among periods as shown in Table (7).

All students in the period II were within mild to severe range of stress, while in the period I and III were within normal range of stress. Chi-square test was showed that there was HS difference at P-value ≤ 0.01 among periods as shown in table (8).The mean of IL1β was elevated in the period II in comparison with the periods I and III as shown in Table (9).ANOVA test was showed that there was HS difference at P-value ≤ 0.01 among and within periods, Table (10). LSD was showed that there were HS differences at P-value ≤ 0.01, Table (11). Pearson's correlation coefficient (R) of salivary IL-1β with clinical periodontal parameters (PLI, GI and BOP) and stress were strong correlations and HS differences at P-value ≤ 0.01 in all parameters, except BOP score (0) the correlation was inverse (negative) correlation and NS difference at P-value ≥ 0.05 as shown in Table(12) and Pearson's correlation coefficient of stress with salivary IL-1β and clinical periodontal parameters were strong correlations and HS differences at P-value ≤0.01 in all parameters, except BOP score (0) the correlation was inverse and NS difference at P-value ≥ 0.05 as shown in Table (13).

DISCUSSION

The results of this study involving dental students because recent studies have reported high levels of stress among dental and medical students [15,17,18], aged 21-23 years of the fifth class, we selected this class because final class represent more stress class.Over the past decade, it has become more apparent that stress can negatively influence oral health status, which can lead to increased amounts of dental plaque, gingival inflammation and more severe periodontitis[19, 20]. In the present study, the mean value of PLI and GI of period II was significantly higher than that of periods I and III, students were found to have more plaque accumulation and gingival inflammation during an exam period, suggesting that examination stress might influence periodontal health status. This result is in conformity with many previous studies [15, 21, 22, 24], who found increased dental plaque and gingival inflammation in students who experienced examination stress. The increased levels of dental plaque and gingival inflammation in the present study may be explained either by influence of increase plaque accumulation during the exam period leading to gingival inflammation, as the plaque is the causative factor of gingival inflammation (behavioral model), behavioral changes in the stressed students, for example, inattention leads to oral hygiene mechanism might be less effective and ⁄ or reduced in frequency during this time of stress, this changes occurring in behavioral have been considered as a reason for association between stress and gingivitis, orby the direct influence of stress on immune system (biologic model), both resulting in increased susceptibility to PD [25].After the exam period, a reduced amount of dental plaque was found and this may partly be explained by the Hawthorne effect [23], meaning that panelists involved in clinical trials might be affected because of attention and interest.The percentage of sites with BOP was significantly higher in period II than periods I and III. The potential altered abilities of period II to perform effective oral hygiene could result in an increased BOP that exacerbates the risk for enhanced tissue destruction in PD. Moreover, interesting observations regarding the complexity of the oral and systemic challenge provide unique mechanisms by which deregulation of host responses could occur due to immunologic and behavioral changes, related to examination stress may be lead to PD. Where examination stress appears to affect periodontal health status, shown by more plaque accumulation, gingival inflammation and increased amounts of proinflammatory cytokine salivary IL-1β therefore examination stress appears as a possible risk factor for gingivitis. This result is in agreement with many previous studies [15,21,24].The students registered their perceived stress on DASS-21. The DASS is a simple-to-administer, reliable and a valid measurement tool for evaluating stress [17]. The DASS summarized magnitude of psychological derangement by summing the scores obtained from stress domain only from this scale. Where students were asked how stressed they felt on a 4-points scale for all periods to compare between them. According to the results of this study, the percentage of stress was significantly higher in period II than period I and III. Where the DASS scores were significantly higher during the exam period compared with other periods. This result is

agrees with **Johannsen et al.** [15] which used a visual analogue scale to register the perceived stress of students during an exam period compared with after the exams, **Michael et al.**[26], which used DASS to psychological distress in diurnal variations, **Singh et al.**[27], which used DASS in measuring stress and its effect on cortisol level in medical student and **Premkumar et al.**[28], which used DASS in measuring changes in mood. The proper explanation for this result was dental students who participated in an exam had significantly more stress compared with students who didn't participate in any exam.Salivary IL-1β was used in this study instead of serum IL-1β because saliva has been used as a diagnostic biofluid to measure host responses to a variety of triggering factors in systemic and oral diseases [29]. Saliva analyses have advantages of quick and easy sample collection not requiring specialized equipment or personnel. Moreover, its sampling is painless and noninvasive, therefore, saliva sampling doesn't cause stress to students. It's one of the most promising mediums for its diagnostic potential for various diseases including stress-related diseases, and it's readily available any time and for repeated samplings [30].Also, IL-1β levels are generally higher in saliva than in plasma or serum, and serum/plasma levels are often below the limit of detection [31, 32].IL-1 is important pro-inflammatory cytokine in the pathogenesis of PD [10]. It induces widespread gene expression of cyclooxygenase-2, inducible nitric oxide synthesis and MMP, which results in activation of osteoclasts, bone resorption and down regulation of type I collagen expression in bone [33]. Although both isoforms of IL-1 (1L-1α and IL-1β) have similar biological activities and appear to be contributory, but IL-lβ is more potent in stimulating bone resorption and is the form more frequently occurring in periodontitis [34]. In addition IL-1β is a critical mediator of adaptive stress responses as well as stress-associated neuropathology and psychopathology [11]. For all these reasons, salivary IL-1β was used in this study and the current study is the first of its kind in Iraq, that reflect the association between stress, salivary IL-1β and periodontal health status;the mean value of IL-1β of period II was significantly higher than that of the period I and III. Where IL-1β stimulates the HPA axis activity and associated with immune system and inflammation response during stress [11]. This result is agrees with many previous studies [24, 35], who found higher amounts of IL-1β in GCF during academic examination stress and **Brydon et al.**[36], who found higher amounts of IL-1β in human mononuclear cells during psychological stress and disagrees with **Marques-Deak et al.**[37], who reported similarities of IL-1β level in both stressed and non-stressed individuals and **Johannsen et al.** [16], who reported non-significant difference of IL-1β level between during and after exam. One problem in stress studies in general could be the difficulty to know when the influence on the biomarkers by a stress period is over. An explanation to why **Marques-Deak et al.**[38] and **Johannsen et al.**[15] didn't find high IL-1β levels in GCF, despite a high degree of inflammation could be that hormonal stress inhibits IL-1β response to stress (feedback regulation of IL-1β) [38, 39].Also, some studies have shown that level of pro-inflammatory cytokine, IL-1β, are increased in patients with depression [20, 40]; however, contradictory results have also been described [41].IL-1β level is a sensitive and reliable marker of chronic inflammatory disease activity and IL-1β elevation may demonstrate tissue destruction [42]**.** Thus, in this study the detection of elevated levels of IL-1β in saliva of subjects with stress was consistent with the cytokine's role in inflammation and suggests that salivary lL-1β may be a good marker of periodontal inflammation.There was a strong positive correlations between stress, IL-1β and clinical periodontal parameters.These results were in agreement with **Maes et al.** and **Waschul et al.** [43, 44], where stress alters immune function, hence increase production ofpro-inflammatory cytokine (humoral immunity) and decrease cellular immunity that lead to periodontal inflammation.In addition, stress was related to PD because psychological stress can directly affect periodontal health status by various biological (physiological) mechanisms; also, it can have indirect effects through the behavioral (psychological) changes in lifestyle such as ignoring oral-hygiene measures that lead to increased levels of dental plaque and consuming more sugar in diet [45]. PD develops during stressful condition by tissue destroying factors as IL-1β activated by the direct effects of pathogenic bacteria in dental plaque. In addition, many physiopathological processes are involved in periodontal destruction in terms of the inflammatory and immune host response, especially proinflammatory cytokines or MMPs [8, 9]. All these factors lead to strong correlation among IL-1β, plaque, gingival inflammation, BOP and stress. This result is in agreement with many previous studies [24, 35].

As a conclusion, the examination stress was affected on periodontal health status by more plaque accumulation, gingival inflammation and

increased amounts of IL-1β in saliva, so there was strong correlations between themand examination stress was appeared as a possible risk factor for gingivitis.

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Table (1): Descriptive statistical results of plaque index for each period.

Period	Min	Max	Mean	SD
I	0.23	1.14	0.637	0.256
II	0.56	1.40	1.028	0.247
III	0.01	0.73	0.230	0.227

Table (2): ANOVA test for plaque index.

ANOVA	SS	df	MS	F-test	P-value	Sig
Among periods	7.64	2	3.82	64.24	0.000	**
Within periods	4.10	69	0.06			
Total	11.75	71				

P ≤ 0.01 High significant (HS) **

Table (3): LSD test of plaque index between each two periods.

Periods		MD	SE	P-value	Sig
I	II	-0.390	0.070	0.000	**
	III	0.407	0.070	0.000	**
II	III	0.798	0.070	0.000	**

Table (4): Descriptive statistical results of gingival index for each period.

Period	Min	Max	Mean	SD
I	0.03	0.56	0.202	0.168
II	0.20	1.17	0.600	0.270
III	0.02	0.31	0.105	0.084

Table (5): ANOVA test for gingival index.

ANOVA	SS	df	MS	F-test	P-value	Sig
Among periods	3.30	2	1.65	45.47	0.000	**
Within periods	2.50	69	0.03			
Total	5.80	71				

Table (6): LSD test of gingival index between each two periods.

Periods		MD	SE	P-value	Sig
I	II	-0.397	0.054	0.000	**
	III	0.097	0.054	0.081	NS
II	III	0.495	0.054	0.000	**

Table (7): Percentages and No. of scores of BOP for each period and comparison among periods.

Scores of BOP	Period I		Period II		Period III					
	No	%	No	%	No	%	Chi-square	df	P-value	Sig
0 (no bleeding)	2624	97.6%	2508	93.3%	2657	98.8%	138.339	2	0.000	**
1(bleeding sites)	64	2.4%	180	6.7%	31	1.2%				

Table (8): Percentage and No. of stress range and comparison among periods.

Scores of Stress	Period I		Period II		Period III					
	No	%	No	%	No	%	Chi-square	df	P-value	Sig.
Normal (0-14)	24	100.0%	0	0.0%	24	100.0%	72.000	6	0.000	**
Mild (15-18)	0	0.0%	4	16.7%	0	0.0%				
Moderate (19-25)	0	0.0%	15	62.5%	0	0.0%				
Sever (26-33)	0	0.0%	5	20.8%	0	0.0%				
Extremely sever (34)	0	0.0%	0	0.0%	0	0.0%				

Table (9): Descriptive statistical results of IL-1β for each period.

Periods	Min	Max	Mean	SD
I	60.39	674.85	269.920	158.790
II	122.04	1228.07	552.566	275.622
III	39.72	263.96	114.019	72.518

Table (10): ANOVA test for IL-1β level.

ANOVA	SS	df	MS	F-test	P-value	Sig
Among periods	2372144.09	2	1186072.04	33.42	0.000	**
Within periods	2448150.21	69	35480.43			
Total	4820294.31	71				

Table (11): LSD to compare the means of IL-1β between each two periods.

Period		MD	SE	P-value	Sig
I	II	-282.646	54.375	0.000	**
	III	155.901	54.375	0.005	**
II	III	438.547	54.375	0.000	**

Table (12): Correlation of salivary IL-1β with PLI, GI, BOP and stress among periods.

	Salivary IL-1β		
	R	P-value	Sig
PLI	0.54	0.000	**
GI	0.50	0.000	**
BOP score (1)	0.41	0.000	**
BOP score (0)	-0.09	0.450	NS
Stress level	0.66	0.000	**

Table (13): Pearson's correlation of stress with IL-1β and PLI, GI, BOP among periods.

	Stress		
	R	P-value	Sig
PLI	0.71	0.000	**
GI	0.68	0.000	**
BOP score (1)	0.54	0.000	**
BOP score (0)	-0.01	0.450	NS
Salivary IL-1β	0.66	0.000	**