Assessment of Salivary Total Antioxidants Capacity Levels of Patients with Chronic Periodontitis in Comparison to Healthy Control

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ABSTRACT

Background: Chronic periodontitis (CP) is greatly prevalent condition of inflammatory behavior. Salivary biomarker total antioxidants capacity (T-AOC) status, may be related to both periodontal condition and oral hygiene.

Aims of the study: To assess the level of salivary T-AOC of patients with chronic periodontitis in comparison to healthy control and to correlate between the level of this marker with the clinical periodontal parameters (plaque index (PLI), gingival index (GI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL)).

Materials and Methods: Ninety subjects of males and females with an age ranged between (35-55) years were participated in this study. Participants were divided into two groups: the first group was CP group that consisted of fifty-five subjects and the second group consisted of thirty-five subjects as control group with healthy periodontium and both groups systemically healthy.

The whole unstimulated salivary samples were collected, and then periodontal evaluation that including the assessment of clinical periodontal parameter (PLI, GI, BOP, PPD, and CAL) were done for all participants. Enzyme-linked immune– sorbent assay (ELISA) used to determine the level of T-AOC in saliva.

Results: The two studied groups showed a highly significant difference regarding the salivary level of T-AOC, and it revealed that the mean value of salivary level of T-AOC was statistically lower in CP group than the control group. Regarding Pearson Correlation Coefficient, this study revealed that there is strong negative correlations between clinical periodontal parameters (GI, BOP, PPD, and CAL) with salivary level of T-AOC.

Conclusion: Salivary T-AOC could be used as a reliable marker of chronic periodontitis activity.

Keywords: periodontal disease, total antioxidants capacity. **(J Bagh Coll Dentistry 2018; 30(1): 58-62)**

INTRODUCTION

Periodontitis is a progressive, multifactorial disease, and associated with inflammation. It can be described by the pathogenic bacterial colonization, and the advancement of alveolar bone and connective tissues destruction that leads to probable tooth loss [1]. Reactive cells, when stimulated by pathogenic bacteria and their associated cell membranes lipopolysaccharides, produce cascade of cytokines potentially responsible for destruction of the periodontal tissue [2].

Subgingival biofilm acts as a main factor in the periodontal disease pathogenesis by the stimulation of immune responses that can result in destruction of periodontal tissue [3, 4]. Additionally, vulnerability to periodontal disease, along with its severity and advancement, are affected by genetic, environmental, and acquired risk factors that can make modification in host reactions [5, 6].

Oxidants that formed during inflammatory process either interact with target proteins or neutralized by antioxidants system. So, salivary T-AOC measurement can be considered as an important periodontal diagnostic tool[7].

The T-AOC is a combined biomarker that reproduces the collective action of primarily non-enzymatic antioxidants existing in the body fluids and plasma [7, 8]. It is advised that the T-AOC measurement may offer facts on the equilibrium between antioxidant and oxidants systems [9].

An insufficient antioxidant capacity may have a role in the increase tissue damage [9]. Several data proposes an association between T-AOC in saliva and CP, and T-AOC measurement appears to be dependable method that can offer a new and applied method to define the periodontal disease-associated oxidative status [10, 11].

MATERIALS AND METHODS

The participants in this study were consisted of 90 subjects, aged between 35-55 years old from both genders. The human samples were collected from the patients who attended to the dental unit in Bader health center in Al-Kut city. Collection of samples continued from the period between December, 2016 and March, 2017. Informed consents have been assigned by all participants after they had been informed about the aims of the study. We certify that this study involving human subjects is in accordance with the Helsinki declaration of 1975 as revised in 2000 and that it has been approved by the relevant institutional Ethical Committee.

Participants were grouped into two groups:

(1) Master student, Ministry of Health
(2) Professor, Department of Periodontics, College of Dentistry, University of Baghdad.

1. Chronic periodontist (CP) group; It consisted of fifty-five systemically healthy patients with CP which was defined by the presence of at least four sites with probing pocket depth ≥4mm with clinical attachment loss ≥1-2mm [12, 13].
2. Control group; It consisted of thirty-five of non-smoker or pregnant subjects with healthy systemic status and clinically healthy periodontium.

After initial periodontal examination, unstimulated whole saliva was collected according to Tenovuo 1994 [14]. The subject drool the saliva passively in 10 ml centrifuge tube to collect 5 ml of saliva, and the sample was placed in the cooler box to be centrifuged later. After saliva has been collected a comprehensive periodontal examination was done to record clinical periodontal parameters which included:

1. Amount of soft deposits was assessed according to Plaque Index (PLI) by Silness and Loe in1964 [15].
2. Gingival inflammation was assessed according to the criteria of gingival index (GI) system by Loe in 1967 [16].
3. Bleeding on probing assessment according to Carranza et al.[13].
4. Assessment of Probing Pocket Depth (PPD).
5. Assessment of Clinical Attachment Level (CAL).

Afterward the salivary sample is centrifuged at 3000 r/min for 20 minutes then preserved in plane tube and stored in -20°C freezers to be analyzed later by Enzyme Linked Immunosorbent Assay (ELISA) kit for determination of salivary levels of T-AOC. The laboratory tests were done in Laboratories of Al-Kut Hospital.

Statistical analysis was done using mean, Standard Deviation, Standard Error, Minimum, Maximum, percentages, Levene's test, t-test and Pearson correlation coefficient test (r).

The p-value is significant at less than 0.05, highly significant at <0.01, and non-significant at >0.05.

RESULTS

This study showed that the mean value of PLI for the CP group was greater than that of the control group, which were 1.73±0.367 and 0.53±0.238 respectively (Table1). Also the mean value of GI for the CP group was greater than that of the control group, which were 1.87±0.438 and 0.33±0.238 respectively (Table2). The percentage of bleeding sites for CP group was greater than that of the non-bleeding sites (Table 3). Table 4 represented the mean value, Std. Deviation, Std. Error, Minimum, and Maximum of PPD and CAL of the CP group.

In addition, it showed that the mean value of T-AOC for the CP group was greatly lesser than that of the control group, the mean and SD were 29.65±8.960 for the CP group, while they were 68.44±15.657 for the control group (Table 5) and there is a highly significant difference in the T-AOC level between the two groups (Table 6). According to Pearson Correlation Coefficient (r), there is highly significant negative correlation between clinical periodontal parameters [GI (of CP and control groups), BOP, PPD, and CAL (of the CP group)] and salivary T-AOC, while there is non-significant correlation between the clinical periodontal parameter (PLI) of each group and the salivary T-AOC (Table 7).

Table 1: Descriptive statistics of mean values of plaque index (PLI) parameter for the CP and control groups.

Groups	No.	Mean	±SD	SE	Min.	Max.
CP	55	1.73	0.367	0.049	0.76	2.40
Control	35	0.53	0.238	0.040	0.00	0.7

Table 2: Descriptive statistics of mean values of gingival index (GI) parameter for the CP and control groups.

Groups	No.	Mean	±SD	SE	Min.	Max.
CP	55	1.87	0.438	0.059	0.90	2.70
Control	35	0.33	0.238	0.040	0.00	0.5

Table 3: Numbers and percentages distribution of sites according to bleeding on probing (BOP) scores for the CP group

Group		BOP		Total
		Score 0	Score 1	
CP	No	2338	2938	5276
	%	44.313	55.686	100

Table 4: Descriptive statistics of mean values of PPD and CAL for CP group.

	No.	Mean	±SD	SE	Min.	Max.
PPD	55	4.68	1.050	0.141	2.50	7.45
CAL	55	4.18	1.454	0.196	2.40	8.54

Table 5: Descriptive statistics of salivary T-AOC(U/ml) for the CP and Control groups.

Parameter	Groups	No.	Mean	±SD	SE
T-AOC	CP	55	29.65	8.960	1.208
	Control	35	68.44	15.657	2.646

Table 6: Statistical analysis of the mean values of salivary T-AOC for the CP and control groups with comparison of significance.

Independent Samples Test			
Levene's Test for Equality of variance			
F= 28.804	P=0.000		HS
t-test for Equality of Means			
t=-13.332	d.f=48.328	P=0.000	HS

Table 7: Person Correlation Coefficient (r) between salivary T-AOC and clinical periodontal parameter (PLI, GI, BOP, PPD, and CAL) of CP and Control groups.

	Groups	Statistical analysis	PLI	GI	BOP	PPD	CAL
T-AOC	CP	r	-0.073-	-0.457**	-0.504**	-0.464**	-0.572**
		P-value	0.509	0.000	0.000	0.000	0.000
		Sig.	NS	HS	HS	HS	HS
	Control	r	-0.023	-0.591**	-	-	-
		P-value	0.895	0.000	-	-	-
		Sig.	NS	HS	-	-	-
** Correlation is highly significant at the 0.01 level (2-tailed).							

DISCUSSION

The plaque accumulation is the primary cause of periodontal diseases including CP, which is a chronic inflammation of the gingiva and connective tissue [17]. Accordingly, comparison between healthy and CP groups, in term of PLI and GI, revealed great differences in the mean values of these parameters similar to a study done by Khamees et al. [18]. Normally, the healthy control group exhibit no periodontal pockets or attachment loss. In contrast, CP group showed loss of attachment and damage to the surrounding alveolar bone in response to increasing amount of accumulated plaque and bacterial invasion [19]. Therefore, no comparisons were made in BOP, PPD, CAL between the study and control groups.

The salivary total antioxidants concentrations were lower in CP group as compared with the control group and there is a highly significant difference between the studied groups. A highly significant but negative correlation has been found between clinical periodontal parameters (except PLI) and T-AOC and this findings are in consistent with other studies showed that the periodontitis severity is independently correlated with an increased in oxidative stress and a reduction in antioxidant capacity [20, 21]. A possible explanation for such results that periodontal disease is caused by bacteria that colonize the gingival crevices and periodontal pockets. In response to bacterial colonization, chronic and progressive inflammation triggered [22]. Immune response against periodontal bacteria is linked with an enhanced reactive oxygen species (ROS) production by macrophages and neutrophils. To avoid destruction of host tissue, antioxidants neutralized these ROS, which might leads to decreased T-AOC. Infection and inflammation in periodontal disease show a low-grade systemic inflammatory state, which may decrease the systemic and local total antioxidant capacity and increased oxygen radical activity [20].

In conclusion, T-AOC in saliva could be used as reliable marker of chronic periodontitis activity as its level markedly different between the CP and control groups, this could be a useful measurement during assessment of recovery from periodontal disease as well as could lead to different therapeutic approach.

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الخلاصة

الخلفية:التهاب اللثة المزمن هو حالة مرضية شائعه ذات طابع التهابي. مجموع المواد المضادة للاكسدة ,كما بينت العديد من الدراسات, لها علاقة مع كل من التهاب انسجة ماحول الاسنان والحالة الصحية الفموية.

اهداف الدراسة: تقييم مستوى مجموع المواد المضادة للاكسدة في لعاب المرضى الذين يعانون من التهاب اللثة المزمنة بالمقارنة مع الأصحاء، والربط بين مستوى هذه العلامات مع مؤشرات انسجة ماحول الاسنان السريريه (مؤشر الصفيحه الجرثوميه,مؤشر التهاب اللثه ,مؤشر النزف عند التسبير ,مؤشر عمق الجيوب واخيرا مؤشر فقدان الانسجه الرابطه سريريا)

المواد وطرق العمل: تسعون(٩٠) مشارك من كلا الجنسين ادرجوا في هذه الدراسه ,تتراوح اعمارهم بين (٥٥-٣٥) سنه مقسمين الى مجموعتين: .المجموعه الاولى تضم مرضى التهاب دواعم السن المزمن (عددهم =٥٥)، وتتكون المجموعة الثانية من خمسة وثلاثين شخصا كمجموعة ضابطه وكانوا ذوي لثه صحيه واصحاء سريريا.

العينات اللعابية غير المحفزة تجمع، وبعد ذلك تقييم مؤشرات الحالة الصحيه لانسجة ماحول الاسنان لجميع المشاركين(مؤشر الصفيحه الجرثوميه,مؤشر التهاب اللثه ,مؤشر النزف عند التسبير ,مؤشر عمق الجيوب واخيرا مؤشر فقدان الانسجه الرابطه سريريا). نظام مقياس الانزيم المرتبط الممتز المناعي(الايزا) استخدم لتحديد مستويات مجموعة مضادات الاكسدة في اللعاب.

النتائج: أظهرت المجموعتين المدروستين فرقا معنويا كبيرا في مجموعة مضادات الاكسدة في اللعاب ، حيث كشفت أن متوسط قيمة المستوى اللعابي لمجموعة مضادات الاكسدة كان أقل إحصائيا في مجموعة اللثة المزمنة من المجموعة الضابطة. فيما يتعلق بمعامل ارتباط بيرسون كشفت هذه الدراسة أن هناك علاقة عكسية قوية بين مؤشرات الحالة الصحيه لانسجة ماحول الاسنان (مؤشر التهاب اللثه ,مؤشر النزف عند التسبير ,مؤشر عمق الجيوب واخيرا مؤشر فقدان الانسجه الرابطه سريريا) ومستوى مجموع مضادات الاكسدة في اللعاب.

الاستنتاج: يمكن استخدام القدرة اللعابية الكلية لمضادات الأكسدة كعلامة يمكن الاعتماد عليها لمعرفة النشاط المحطم لانسجة ماحول الاسنان لمرضى التهاب اللثة المزمن.