DOI Number: 10.37506/v14/i1/2020/ijfmt/192968

Assessment of Serum Advanced Glycation End-Product Level and Its Effect on Periodontal Health Status in Type 2 Diabetic Patients with Chronic Periodontitis

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Abstract

Background: The bidirectional relationship between diabetes mellitus and periodontitis was obvious as both of them are considered chronic diseases. The risk of developing periodontitis was reported to be higher in diabetic patients specially with poorly control diabetes mellitus, which in turn can negatively impact glycemic control. Advanced glycation end-products have intertwined relationship with oxidative product; increased in advanced glycation end-products could lead to oxidative stress and vice versa. The aim of current study was to investigate the possibility of using serum levels of (AGEs) for identification of the periodontal pathological condition in periodontitis patients with and without diabetes. **Method:** Twenty healthy individuals (control group), 30 patients with severe chronic periodontitis and 30 poorly controlled diabetic patients with severe chronic periodontitis were included. Full mouth (plaque index PlI, gingival index GI, bleeding on probing BOP, probing pocket depth PPD, clinical attachment loss CAL) were recorded by periodontal probing and serum advanced glycation end-products concentration were assayed using enzyme linked immunosorbent assays. **Results**: A non-significant difference was reported in plaque index PlI, gingival index GI, bleeding on probing between diabetic and non-diabetic patients with chronic periodontitis, while probing pocket depth, clinical attachment loss revealed a significant difference between diabetic and non-diabetic patients. Advanced glycation end-products presented with highest level in diabetic group (26.92) followed by chronic periodontitis group (15.91) then the control group (6.60), however, the correlation was non-significant with periodontal parameters. **Conclusions:** It is possible to use serum advanced glycation end-products level in the early diagnosis of chronic periodontitis in patients with and without diabetes.

Keywords*: chronic periodontitis, advanced glycation end-products, plaque index, gingival index, bleeding on probing.*

Introduction

Diabetes mellitus has a series of metabolic disorders recognized by disorders in insulin action, secretion or both causing hyperglycemic state [(1)], diabetes complications occurs with long periods of poor glycemic control [(2)].

Periodontal diseases are inflammatory diseases caused by infection of the supporting tissues with bacteria [(3)]. In spite of the essential role of bacteria in dental plaque, alone it's not enough for the initiation or advancement of periodontium breakdown. So, host response activation causes irreversible tissue destruction, then inflammation and disease resulted from interaction of these microbiota with immune defenses [(4)].

The gingivitis and periodontitis considered as the most popular forms of periodontal diseases. Gingivitis, a stable form, it is considered a reversible inflammation of the gingiva with no involvement of the attachment apparatus, while periodontitis includes the deeper structure of periodontium leading to loss of attachment with the destruction of periodontal structure [(5)].

Chronic periodontitis (CP) is considered as the most popular type of periodontitis it mostly affects adults between 40 - 50 years old and it is reported as the essential cause of tooth loss. It progresses slowly, but may be subjected to periods of exacerbation [(6)].

The relationship between diabetes mellitus and periodontitis was reported as a bidirectional cyclical relationship [(7)]. Moreover, in several studies the presence

of diabetes was associated with higher incidence, prevalence and severity of chronic periodontitis [4,8]. Thus, CP was counted as the sixth complication of diabetes [7], and diabetes considered as a risk factor for boosting periodontal disease [9]. The risk of developing periodontitis was reported to be three-fold higher in people with poorly-controlled diabetes, which in turn can negatively impact glycemic control [10].

Advanced glycation end products (AGEs) are proteins or lipids that turn into glycated following exposure to sugars. AGEs represent a heterogeneous complex that is produced continuously under physiologic conditions and their production is greatly increased in case of atherosclerosis, hyperglycemia as well as inflammation and oxidative stress [11]. A study conducted in 2018 confirmed the results of previous studies about the intertwined relationship between AGEs and oxidative product (OP) [12]. Furthermore, recent studies confirmed the past evident on the involvement of AGEs in periodontal disease [13] and diabetes complications [14]. All of that motivated us to perform current work in order to study the possibility of using serum levels of (AGEs) for identification of the periodontal pathological condition in periodontitis patients with and without diabetes.

Materials and Method

Study participants

Human samples consisted of eighty males; age range was (35-55) years. Information taken from each participant about the name, age, full dental and medical history, if he was taken any medications, smoking or drinking alcohol, level of HbA1c for diabetic patients and the diabetic period.

The study was approved by the ethical committee of college of dentistry/ university of Baghdad. Each subject was informed about the aims of the investigation according to the informed consent written in simple English and Arabic words, and they were free to choose whether to participate in this study or not. Participants were categorized into three groups: **A.** Poorly-controlled type 2 diabetes mellitus with sever Chronic periodontitis (CP+pT2DM); composed of 30 males with mean clinical attachment loss ≥ 5mm, HbA1c >9% and on oral hypoglycemic medication. **B.** Sever chronic periodontitis (CP) without any systemic disease; composed of 30 males with mean clinical attachment loss ≥ 5mm. **C.** Systemically healthy with clinically healthy periodontium (control group); composed of 20 males seemingly healthy without any systemic diseases with apparently healthy periodontium.

Inclusion and exclusion criteria

The inclusion criteria were 1. patients with generalized severe chronic periodontitis CP must have CAL ≥3mm at >30 % of the sites with mean of CAL ≥5mm [15], 2. At least 16 teeth present, 3. Patients with T2DM for ≥5 years and on oral treatment only, 4. All participants within normal range of body mass index (18.5-24.9kg/m^2). The exclusion criteria were: 1. Females were excluded from the study, 2. Presence of systemic disorders other than T2DM, 3. Patients who were receiving periodontal treatment within three months before the study, 4. Patients with T1DM and T2DM receiving insulin as a treatment, 5. Having a course of antibiotic in three months prior to the study, 6. Medications that may affect periodontal tissue and 7. Drinking alcohol or smoking.

Clinical periodontal parameters

All participants were subjected to full mouth periodontal examination excluding 3rd molar by Michigan O periodontal probe for (plaque index PlI, gingival index GI, bleeding on probing BOP, probing pocket depth PPD, clinical attachment loss CAL) on four surfaces (mesial, buccal/ labial, distal and lingual/ palatal).

HbA1c measurement

A volume of 5ml venous blood was gathered from each individual, for diabetic group 1ml placed into Ethylene diamine tetra acetic acid (EDTA) tube in order to measuring HbA1c and 4ml gathered into a serum separating tube (gel tube) and then centrifuged at 3000rpm for 15 minutes. Serum was divided into Eppendorf tubes then labelled and stored at -50°C till used for analysis.

The measurement of HbA1c for patients with T2DM was done by using Standard A1c Care Test kit analyzer. The procedure was executed according to manufacturer's instructions.

Biochemical analysis of advanced glycation end products (AGEs)

Assessment of AGEs level was performed using ELISA kit (96 wells) for quantitative measurements

of AGEs in serum (CUSABIO AGEs ELISA kit Catalog Number.CSB-E09412h, china) according to manufacturer's instructions.

Statistical Analysis

Data were processed and analyzed using the Statistics Package

for Social Sciences (SPSS; version 22) with both descriptive and inferential statistics. Means were used to express all values. LSD test and *t*-test were used in data analysis to compare mean values among the groups. Correlation between AGEs and clinical periodontal parameters was tested by Person's Correlation Coefficients (r) test. Moreover, in the statistical evaluation, differences were considered significant when probability value $P<0.05$ and $P> 0.01$.

Results

Thirty (CP+pT2DM) patients, thirty CP patients and twenty healthy control individuals were studied. PLI and GI of control group revealed significant differences from (CP+pT2M) and CP groups (Table 1). Moreover, BOP presented non-significant difference, while CAL and PPD showed significant difference from (CP+pT2M) and CP groups (Table2).

Table 1 Intergroup comparisons of the mean values of PLI and GI parameters between all pairs of study and control groups

Parameter	Group		Mean Difference (I-J)	Std. Error	Significance
PLI	**CP+pT2DM**	Control	1.99100*	0.06711	0.000**
		CP	0.03433	0.06002	0.569
	CP	Control	1.95667*	0.06711	0.000**
GI	CP+pT2M	control	1.94100*	0.03893	0.000**
		CP	0.01367	0.03482	0.696
	CP	control	1.92733*	0.03893	0.000**

****: Significant at *P*>0.01.**

Table 2): Intergroup comparisons of mean percentages of score 1BOP, PPB and CAL parameters between study groups

Parameter	Group	No.	Mean	Std. Deviation	t	Significance (2-tailed)
BOP	CP	30	70.40	9.27	-0.827	0.411
	CP+ pT2DM	30	73.01	14.60		
PPD	CP	30	5.24	0.64	-2.349	0.022*
	CP+ pT2DM	30	5.58	0.48		
CAL	CP	30	5.14	0.41	-3.428	0.001**
	CP+ pT2DM	30	5.57	0.55		

***: Significant at *P*> 0.05. **: Significant at *P* >0.01.**

Regarding AGEs, the highest mean value was noticed in diabetic group followed by CP group then the control group (Figure 1). As shown in (Table 3) the AGEs reported significant difference between (CP+pT2M) and CP and control groups as well as between CP and control groups. Furthermore, serum AGEs had a non-significant correlation with all the clinical periodontal parameters (Table 4).

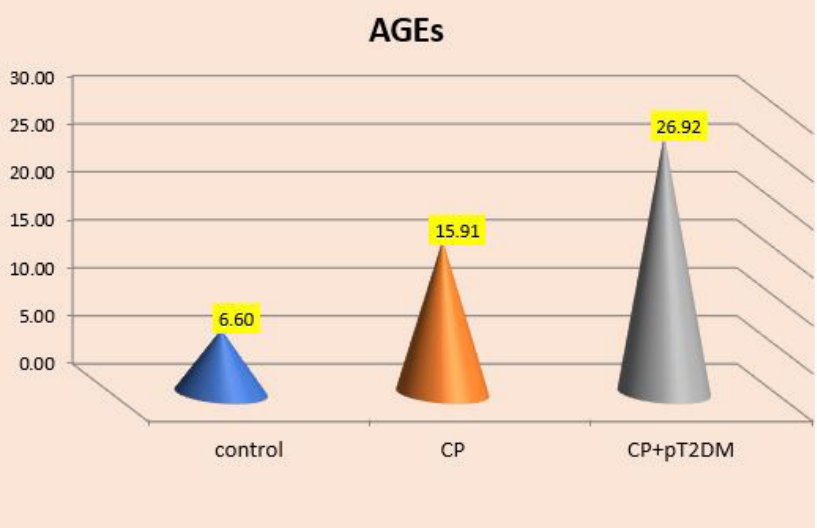

Figure 1 mean serum concentrations of AGEs (ng/ml) for study and control groups.

Table 3): Intergroup comparisons of mean serum concentrations (ng/ml) of AGEs between all pairs of study and control groups

parameter	**groups**		**Mean Difference (I-J)**	**Std. Error**	**Significance**
AGEs	**CP+pT2DM** CP	Control	20.319767	4.473531	0.000**
		11.002300	4.001248	0.007**	
	CP	Control	9.317467	4.473531	0.041*

***: Significant at *P*> 0.05. **: Significant at *P* >0.01.**

Table 4 Correlations between levels of AGEs with the clinical parameters at each study and control groups

Parameters	**StaStatistical analysis**	**CP+pT2DM**	**CP**	**Control**
PLI	r	-0.132	-0.202	-0.113-
	P-value	0.486	0.285	0.635
GI	r	-0.084	-0.331	-0.214-
	P-value	0.658	0.074	0.364
BOP	r	-0.114	-0.219	
	P-value	0.549	0.246	
PPD	r	-0.229	0.192	
	P-value	0.223	0.308	
CAL	r	-0.138	0.024	
	P-value	0.466	0.900	

Discussion

A significant difference in PlI and GI between control group and both of diabetic and non-diabetic groups with CP, while a non-significant difference was reported between (CP+pT2M) and CP groups**.** This could be attributed to the effect of microbial dental plaque since it is considered as the essential factor in the pathogenesis of periodontal disorders [5,16]**,** and its causes gingival inflammation due to its accumulation on teeth with sign and symptom of inflammation (erythema, edema, bleeding, tenderness and enlargement) [17]. Since both groups had been chosen with strict criteria with same degree of severity, this may explain the non-significant difference between study groups, (CP+pT2M) and CP. Moreover, BOP reported a non-significant difference between study groups. This could be attributed to similarity in subgingival microbiota between diabetic and non-diabetic patients [18].

On the other hand, the CAL and PPD revealed significant differences between study groups. This could be attributed to the effect of DM on the production of proinflammatory cytokines, like IL-6, by human gingival fibroblasts which cause an increase in their production when compared to non-diabetic [19]. Also, patients with hyperglycemia and periodontal disease reported an increase in the expression of TLRs in periodontal tissues which cause higher inflammatory response in those patients [20]. Thus, the incidence, prevalence and severity of periodontitis will be higher in diabetic than non-diabetic individuals [21]**.**

The biochemical analysis of serum AGEs demonstrated the highest level in diabetic group followed by CP group and then control group with significant differences between them. This could be explained by the significant association of AGEs with oxidative stress and inflammation [22]. Levels of ROS were reported to be higher in periodontitis patients than healthy controls [23].

On the other hand, poor glycemic control leads to an increase in AGEs accumulation [24,25] and their formation rate was highly increased in case of hyperglycemia and oxidative stress [26]. So, in case of diabetes together with condition of chronic high oxidative stress will lead to further acceleration in the process of formation of AGEs [22].

Regarding the correlation between AGEs and clinical periodontal parameters, although the correlation was non-significant, there was an elevated level of this marker in chronic periodontitis groups compared to healthy control group suggesting its association with periodontitis and this could be explained by the association of AGEs with progression of periodontal disease [26].

Accumulation of advanced glycation end-products (AGEs) in oral tissues including periodontal fibroblasts and gingival tissues and its interactions with their receptors (RAGE) reported to be associated with impaired fibroblastic growth in periodontal tissues [25] and causes an increase in formation and release of ROS from cells of periodontal ligament with subsequent induction of metalloproteinase (MMP) and proinflammatory cytokines. All that will lead to degradation of connective tissue, osteoclast activation and bone loss [27].

Conclusions

The increased level of serum AGEs in CP groups suggested their potential role in initiation or progression of periodontitis in patients with and without diabetes. Also, the higher level of AGEs in CP group compared with control group may raise the potential involvement of AGEs in the etiology and progression of periodontal diseases. Furthermore, it is possible to use serum AGEs level in the early diagnosis of CP in patients with and without diabetes.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

Funding: Self-funding.

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