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ORIGINAL ARTICLE

Comparing the Levels of Gingival Crevicular Fluid Prostaglandin E2 in Generalized Chronic Periodontitis Between Healthy and Type 2 Diabetes Patients: A Case-Control Study

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Abstract

Objective: To compare the prostaglandin E2 (PGE2) levels of gingival crevicular fluid in generalized chronic periodontitis between healthy and type 2 diabetic patients. **Material and Methods:** 56 diabetic and non-diabetic participants with generalized chronic periodontitis were selected randomly. They were divided into two groups (G1: generalized chronic periodontitis patients with normal blood sugar; and G2: generalized chronic periodontitis patients with diabetes). Gingival crevicular fluid samples were obtained from both groups. The average of 2 samples per day were centrifuged in a laboratory at 2500 rpm and temperature of 4°C for 5 minutes and placed in a refrigerator at -20°C. The level of PGE2 was measured using ELISA and Abcam kit. Data were analyzed by Kolmogorov-Smirnov, Mann-Whitney U Test, Pearson and independent T tests. The significant amount was considered 0.05 in this test ($\alpha<0.05$). **Results:** The mean level of PGE2 was significantly different in the two groups and the mean level of PGE2 in the control group was lower than the case group. There was no statistically significant relationship between PGE2 with pocket depth, fasting blood sugar (FBS) and HBA1C ($p>0.05$). **Conclusion:** PGE2 level of diabetic patient group with chronic generalized periodontitis was significantly more than non-diabetic group with generalized chronic periodontitis.

Keywords: Periodontal Diseases; Chronic Periodontitis; Diabetes Mellitus.

Introduction

Periodontitis is a periodontal inflammation that destroys connective tissue attachment of the tooth. Periodontitis can be occurred in three main forms: chronic, aggressive and as a manifestation of a systemic disease. Chronic periodontitis is the most common form of this disease and generalized form involves more than 30% of dentition [1].

The condition is started by pathogenic microbes in dental plaque. The microbial challenge by the sub gingival biofilm causes recruitment of immune cells into the periodontium, which produces cytokines and inflammatory mediators. Several types of prostaglandins (as PGE2) are known inflammatory mediators implicated in periodontal disease pathogenesis. PGE2 causes vasodilatation, increased vascular permeability and consequently regulate the production of osteoclast activating factor thus facilitating bone destruction [2].

PGE2 levels, which is identified in gingival crevicular fluid (GCF) of patients with periodontal disease, is higher than periodontally healthy subjects and also PGE2 concentration in GCF is valuable for predicting periodontitis progression. PGE2 levels in human periodontal disease using radioimmunoassay was found 10 times greater than of PGE2 in diseased as compared to healthy tissue [3].

Diabetes is a metabolic disorder characterize by chronic hyperglycemia reduced insulin production, impair insulin act, cause breakdown of glucose transfer from blood into the tissues, which sequentially results in high blood glucose levels. Change in the host immunoinflammatory reaction to pathogens plays a major role. Diabetes may cause damage adherence, chemotaxis and phagocytosis of neutrophil, which ease bacterial persistence in the periodontal pocket and considerably increase periodontal damage [4]. There is evidence that suggests that dysregulation of prostaglandin contribute to delay in wound healing [5].

In recent study, type 1 diabetics and non-diabetics were compared and it was established that the PGE2, IL-1β and TNFα levels in GCF were 2-3 times more in diabetics than in non-diabetics [6]. The high PGE2 and IL-1β in GCF of type 1 diabetic patients may be a result of a systemic reaction and manifestation of gram-negative infections such as periodontal diseases can cause more severe periodontal condition [7].

Since no study similar to present research has ever been conducted in Iran and considering the fact that both diabetes and periodontitis are multifactorial diseases, which can be affected by many factors such as genetics, hence the purpose of present case control study was to investigate PGE2 levels in gingival fluid in individuals with periodontitis and diabetes.

Material and Methods

Study Design

The case-control study was accompanied at the Department of Periodontology of Dental Faculty of Tabriz University of Medical Sciences during nine months from October (2015) to June, (2016).

Study Population

The target population included 56 persons suffering from chronic generalized periodontitis with and without diabetes. In choosing the patient's attention was taken for the existence of periodontitis, not to have any systhemic problem excepting diabetes mellitus type 2, to have minimum ≥5mm periodontal pocket depth and minimum of 15 teeth, not to have an antibiotic and antiinflammatory drugs and periodontal therapy in last 6 months.

Any periodontal treatment was not done to sampling site so it would not affect the present periodontal status. Also no recomendation was given to modifey their oral care. Periodontal Pocket Depth (PPD) was determined by using a Williams probe. All measurements were done on all teeth at 6 sides (distobuccal, buccal, mesiobuccal, distolingual, lingual and mesiolingual) and documented as millimeter.

Collecting Gingival Crevicular Fluid (GCF)

Gum tracheal fluid of patients was collected from diabetic and non-diabetic patients. Relative isolation was carried out with cotton roll. Two minutes after ensuring isolation, the sampling of oral salivary was carried out by placing the paper cone #25 in sulcus at a depth of 3 mm for each selected case with 15-19 paper cones in the area and 3 minutes later paper cones were removed and the paper cones that were contaminated with blood were removed from samples (due to the inflammation of the sampling area, more paper cones were contaminated with blood and an average of 11 paper cones were transferred to the vials) and the rest of paper cones containing GCF were placed inside the phosphate buffer saline (PBS) in each individual vial and the gingival fluid was transferred to the vial through shaking of the paper cones inside PBS. At the end of each day, the average of 2 samples per day were centrifuged in a laboratory at 2500 rpm and temperature of 4°C for 5 minutes and placed in a refrigerator at -20°C until all samples were collected for final examination. After taking samples, the PGE2 level was measured using ELISA and Abcam method (ab133021 Prostaglandin E2).

Data Analysis

Data were analyzed using IBM SPSS Statistics for Windows software, version 15 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate the absolute and relative frequencies, mean and standard deviation. Kolmogorov-Smirnov and Pearson tests along with t-test for independent sample or its non-parametric equivalence Mann-Whitney U Test were used in the analyis. In this study, p-value less than 0.05 was considered statistically significant.

Ethical Aspects

This research project was approved by the Ethics Research Committee of the Tabriz University of Medical Sciences (Protocol No. IR.TBZMED.REC.1395.452). All participants signed an informed consent form.

Results

There was no statistically significant relationship between gender of participants and the study groups (p<0.05) (Table 1).

Table 1. Distribution of participants according to group.

Groups	Gender	
	Male N (%)	Female N (%)
Case	15 (57.7)	11 (42.3)
Control	12 (46.2)	14 (53.8)

The Pearson test was used to evaluate the probability of significant statistical relationship between PGE2 in case group and pocket depth, FBS and HbA1C of case group (Table 2). There was no statistically significant relationship between PGE2 in case group and pocket depth, FBS and HbA1C of case group (p > 0.05).

Table 2. Distribution of the Pearson test values.

Case Group	Pearson Correlation	p-value
Periodontal Pocket Depth & PGE2	0.143	0.486
FBS & PGE2	0.054	0.793
HbA1C & PGE2	0.199	0.330

The mean and standard deviation of PGE2 variable have been represented in Figure 1. According to the results, the mean PGE2 level was higher in the case group compared to the control group.

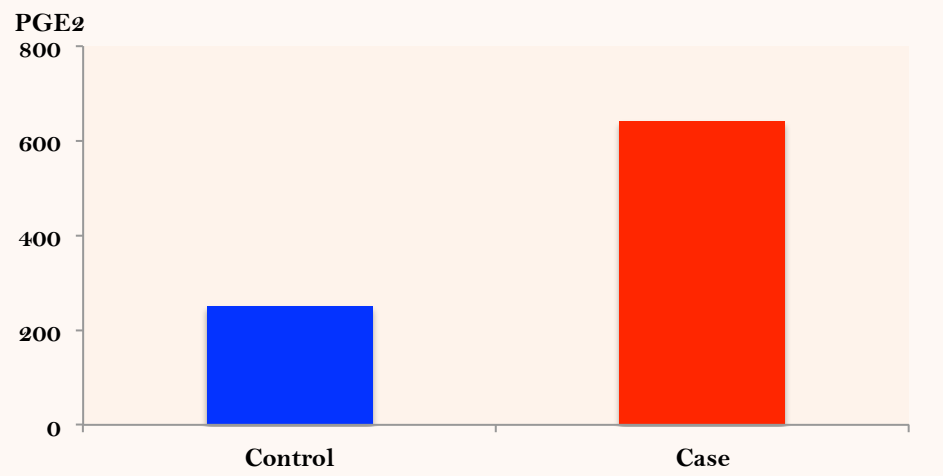

Figure 1. Mean curve of PGE2 in case and control groups.

There was no statistically significant relationship between periodontal pocket depth variable means in two study groups (p>0.05). There was a significant difference between PGE2 means of two study groups and the mean of PGE2 was higher in case group compared to the control group (p<0.001) (Table 3).

Table 3. Comparision of the studied variables in case and control groups.

Variables	Control Group Mean (SD)	Case Group Mean (SD)	p-value
Periodontal Pocket Depth	3.65 ± 0.63	4.14 ± 0.87	0.057
HbA1		8.22 ± 2.13	
FBS		215.96 ± 124.36	
PGE2	251.31 ± 223.12	640.48 ± 375.58	<0.001

Discussion

There are some studies about the relation between diabetes mellitus and increase of oral diseases [8,9]. The research data established that the higher level of risk of development of periodontitis is expected in the diabetic patients whose metabolic control is poor and the destruction level is higher [10,11].

Similarly, there are also many studies targeted to determine the periodontal status and the severity of the periodontal disease in diabetes mellitus patients [8,12-15]. It is showed by many researches that the GCF of type 1 diabetes mellitus patients contains more PGE2 levels than non-diabetic patients [6,16,17].

In this study, comparing PGE2 levels in both diabetic and non-diabetic groups showed that there was a significant difference between them, in a way that the PGE2 level was higher in diabetic people, being in agreement with previous findings [6,16] who recommended that the GCF PGE2 levels of type 1 diabetes mellitus patients with periodontitis were significantly higher than the non-diabetic periodontitis patients. A study on investigating of PGE2 and IL-1β and TNFα in diabetic periodontal patients showed that diabetes significantly increases the level of PGE2 and IL-1β and TNFα from monocytes compared to the non-diabetic patients [6]. It was demonstrated that diabetes as a chronic inflammatory disease leads to increased levels of inflammatory cytokines such as PGE2 in the bloodstream [18].

The effect of cyclooxygenase 1 and 2 (COX-1 and COX-2) has been investigated and led to this fact that FBS significantly increases the production of PGE2 [19]. In the present study the results of investigating the correlation between PGE2 with periodontal pocket depth, HbA1C and FBS in the case group showed that there was no correlation between PGE2 level with the periodontal pocket depth, HbA1C and FBS. In the contrast, a study on evaluating of the gingival fluid in people with oral and systemic diseases concluded that there is higher level of PGE2 in gingival fluid of people with type 2 diabetes whose level of HbA1C is more than 8% compared to diabetics with HbA1c levels below 8% [20]. The inconsistency of results obtained previously [20] and the findings of present study can be due to the sample differences. While a study used people undergoing periodontal surgery [20], in the present study we analyzed people with chronic generalized periodontitis.

No significant relationship was previously observed between PGE2 and HbA1C levels [21]. Prostaglandin E2 level of gingival fluid with pocket depth in periodontal patients showed that PGE2 level has a significant relationship with periodontal pocket depth and is increased with its increasing

[22,23]. The results obtained with Chinese patients [22] are different from those described in this study. Again, the difference between study samples can be also the reason of this inconsistency. Chinese patients with different periodontal pocket depths were investigated [22], while we have investigated the effect of pocket depth on the level of prostaglandin E2 in people with chronic generalized periodontitis with diabetes and all with high periodontal pocket depth.

The level of gingival fluid PGE2 in type 2 diabetic patients with periodontitis showed that diabetes is a risk factor for periodontal disease and there is a correlation between gingival fluid PGE2 and periodontal disease severity [24].

Strong association between the PGE2 in GCF levels and severity of chronic periodontitis has been investigated [25]. The highest concentration of PGE2 in GCF ranged between (7001-8000 pg/ml) was found in patients diagnosed with generalized severe chronic periodontitis with a percentage (13.3%), while 3.3% of patients diagnosed with localized moderate chronic periodontitis have the lowest concentrations of PGE2 in GCF, which ranged between (1000-2000 pg/ml) [25]. Recently, some studies showed the effect of several drugs (such as melatonin, cyclic NSAID) in reduction of PGE 2 for the treatment of periodontitis in healthy and diabetic patients [26-30].

Based on our study, since the type 2 diabetes mellitus is a risk factor in the diabetic patients, their periodontal control should regularly be done. As an important finding, asymptomatic periodontal diseases, which are understood among diabetic persons, can simply be identified. This study, which compared the two groups, is not able to propose significant relative between PGE2 and action of periodontal disease. Further prespective investigations are necessary to study the relationship between the PGE2 in GCF and other inflammatory mediators in patients with chronic periodontitis. More research should be conducted in the mechanism by which PGE2 causes destruction of periodontal tissue in chronic periodontitis.

Conclusion

It was determined that the GCF PGE2 level of healthy group is significantly lower than diabetes mellitus type 2 group. In this study, there was no statistical relation between the clinical parameters and the PGE2 level.

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Conflict of Interest: The authors declare no conflicts of interest.

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