ADVANCED GLYCATION END PRODUCTS AND THIOBARBITURIC ACID REACTIVE SUBSTANCE IN GINGIVAL TISSUES OF DIABETIC AND NON–DIABETIC PATIENTS WITH CHRONIC PERIODONTITIS

B. HOOSHMAND,¹ M.T. GOODARZI,² F. OURAI,¹ M.R. SAFARI,² AND S. AMIRI³

From the ¹Dept. of Periodontology & Implantology, Dental School, Hamadan University of Medical Sciences, Hamadan, the ²Dept. of Biochemistry and Nutrition, Medical School, Hamadan University of Medical Sciences, Hamadan, and the ³Dept. of Epidemiology, Health School, Hamadan University of Medical Sciences, Hamadan, Iran.

ABSTRACT

Background: Production of advanced glycation end products (AGEs) is directly linked to the level and duration of hyperglycemia in diabetic patients. Oxidative stress plays a major role in the pathogenesis of diabetes mellitus. Free radicals are formed in diabetes by glucose oxidation, nonenzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins. Thiobarbituric acid reactive substance (TBARS) is a factor evidence in the presence of oxidative stress as a potential mechanism underlying periodontal disease associated with diabetes.

Methods: 11 subjects (mean age 38.9 years, 6M, 5F) with chronic periodontitis associated with diabetes (5 Type I, 6 Type II) and 16 subjects (mean age 36.7 years, 7M, 6F) with chronic periodontitis as a matched control group participated in this study. Clinical attachment loss and bleeding on probing were determined in all subjects during clinical examination. FBS and HbA1c were measured in all subjects. Sections of gingival tissue of all patients were removed during periodontal surgery. AGEs and TBARS were measured in all removed gingival tissues. The statistical analysis was carried out using T-test, Mann-Whitney U-test and Spearman correlation coefficient.

Results: FBS in diabetic and non-diabetic patients was 155.0 ± 82.0 and 87.4±10.6 mg/dL respectively and the difference between the two groups was statistically significant (p= 0.03 ). There was also a significant difference in HbA1c between the two studied groups (5±0.04 and 9.1±1.03%) in diabetic and non-diabetic subjects respectively, (p= 0.000). A higher level of TBARS was observed in diabetic patients compared to non-diabetics (1.13±0.3 vs 0.05±0.01 mole/lit ; p= 0.001). Clinical attachment loss also was higher in diabetic patients (p= 0.008).

Conclusion: From the results of this study it can be concluded that oxidative stress plays a major role in the development of periodontitis in diabetic patients.


Keywords: Diabetes, Periodontitis, Advanced glycation end products (AGE), Thiobarbituric acid reactive substance (TBARS).

Corresponding Author: Behzad Hooshmand, Dental School, Hamadan University of Medical Sciences, Hamadan, Iran.

Tel: 0811-4223032, Fax: 0811-4223033, Mobile : 09188125790, E.mail: behzadhoshmand-@yahoo.com

/141
INTRODUCTION

The major complications of diabetes include retinopathy, neuropathy, cardiopathy, altered wound healing and nephropathy. Periodontal disease was recognized as the sixth complication of diabetes. The pathogenesis of diabetic complications is not fully understood. Hyperglycemia in diabetic patients leads to formation of irreversible molecules called advanced glycation end products (AGEs). Evidence suggests that AGEs accumulation in the plasma and tissues of diabetics alters cellular structure and composition, resulting in the complications of diabetes.

Also there is increasing evidence in both experimental and clinical studies that suggest that oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins. Periodontal disease is a group of related, generally chronic inflammatory diseases of the supporting tissues of the teeth that leads to the destruction of the periodontium, which consists of alveolar bone, periodontal ligament, gingiva and cementum. It has been considered that the subgingival microflora may be altered in diabetic patients when compared with non–diabetic patients. There is evidence that the subgingival microflora of type 1 and type 2 diabetic patients is not specific or unique when compared to non–diabetic patients. A relationship has been reported between poor glycemic control and periodontal diseases. Some investigators indicated that type 1 diabetics have increased risk of developing periodontal disease with age and the severity of periodontal disease increases with duration of diabetes. These studies showed that significantly more attachment and bone is lost in type 1 diabetics who have poor glycemic control than in those who are well–controlled or non–diabetics. Furthermore, poorly controlled diabetics have a more severe disease with an increased risk of progressive bone loss.

Some studies carried out on the Pima Indian population in Arizona who have an unusually high prevalence of type 2 diabetes indicate that they have a higher prevalence of periodontal disease. Turkish NIDDM patients also had more severe periodontal disease than non-diabetics. Yan et al. demonstrated that incubation of endothelial cells or infusion of mice with AGEs results in the generation of oxidant stress in a wide range of organs. Indicating the potential correlation between oxidant stress and ultimate tissue damage, they concluded that AGEs deposit in diabetic gingiva to an accelerated degree compared with controls. The generation of an oxidant stress, a process which initiates a cascade of events resulting in vascular dysfunction, is likely to be a critical element involved in the pathogenesis and progression of periodontal disease. These findings support the idea that AGEs can induce oxidative or oxidant stress within the organism and they are measured by produced malondialdehyde, a product of lipid peroxidation.

The aim of the present study was to show further evidence in the presence of oxidative stress as a potential mechanism underlying periodontal disease associated with diabetes. This was carried out by measuring AGEs and TBARS levels in the gingival tissue of diabetics and non–diabetic patients with periodontitis and also sharing the difference in these factors between the studied groups.

MATERIAL AND METHODS

Study groups

After obtaining informed consent, a comprehensive periodontal exam was performed on 11 subjects (mean age 38.9, 6 males and 5 females) with chronic periodontitis associated with diabetes (6 type II, 5 type I) and on 16 subjects (mean age 36.7 years, 7 males and 6 females) with chronic periodontitis as matched controls. Diabetic patients with periodontitis were selected from patients’ referring to the Diabetes Center of Hamadan University of Medical Sciences (west of Iran) and the control group who did not have diabetes and other systemic disease were selected from patients referring to the Clinic of the Dental School or private dental offices. Smoker subjects and patients with any inflammatory disease were excluded from the study.

Clinical attachment loss and bleeding on probing was determined in all subjects during clinical examination.

Specimen collection and testing

A fasting blood sample from all subjects was prepared to measure FBS. A separate sample of blood was also collected to measure HbA1c. FBS was measured using an enzymatic method and HbA1c using thiobarbituric acid method. Sections of gingival tissue of all patients were removed during periodontal surgery. After washing the tissue with cold saline, they were kept in 2 mL of KCl (1.15%) at -20°C to analyze.

Thiobarbituric acid substances assay

Tissue homogenates were prepared by suspending the samples in 1.15% KCl and homogenized. TBARS were measured using a method described by Schmidt et al. The protein concentration of all sample was determined and TBARS in tissue were standardized based on protein concentration.

142}
Table I. FBS and HbA1c levels in diabetic and non–diabetic periodontitis patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>FBS (mg/dL)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non – diabetics</td>
<td>87.4±10.6</td>
<td>5.0±0.04</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>155.0±82.0*</td>
<td>9.1±1.03**</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p= 0.029 , **p= 0.00

**AGEs assay**

Advanced glycated end products were measured in all samples using a calorimetric method described by Schmidt. Statistical analysis was carried out using t-test and/or Mann-Whitney U-test. Spearman correlation coefficient was calculated to show the relationship between different parameters.

Table II. AGEs and TBARS content of gingival tissue in diabetic and non–diabetic periodontitis patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>AGEs (μHMF/g protein)</th>
<th>TBARS (mole/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non – diabetics</td>
<td>29.3±19.9</td>
<td>0.046±0.014</td>
</tr>
<tr>
<td>(n=13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetics</td>
<td>46.7±15.7*</td>
<td>1.13±0.31**</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p= 0.034 , **p= 0.000

**RESULTS**

All studied subjects had bleeding on probing during examination that showed presence of active periodontitis. Mean of clinical attachment loss (CAL) was 3.38 ± 1.2 and 4.75±0.85 (mm, mean± SD) in non-diabetic and diabetic patients, respectively (Table III). This parameter was significantly higher in the diabetic group (p= 0.008). According to the observed BOP and CAL, all subjects had moderate to severe active periodontitis.

FBS and HbA1c levels in all subjects were determined and are summarized in Table I. As these data show FBS and HbA1c were higher in diabetic patients (p= 0.029 and 0.00 respectively).

The AGEs and TBARs in gingival tissue of diabetic and non–diabetic patients are shown in Table II. The AGEs level in gingival tissue of diabetic patients was higher than those of non – diabetic patients and the difference was statistically different (p= 0.034).

Comparing the TBARs level in gingival tissue between the two groups indicated a higher level in diabetic patients (p= 0.01).

A positive correlation was observed between CAL and TBARS in diabetic patients (r= 0.55, p= 0.04) (Table III). Also there was a positive correlation between CAL and AGEs in this group (r= 0.59, p= 0.05).

**DISCUSSION**

The presence of AGEs in the plasma and vascular wall in diabetic patients has been hypothesized to contribute to the development of microvascular disease. AGEs, whose production is directly linked to the level and duration of hyperglycemia, impart an oxidant stress to the organism. Enhanced oxidant stress has been proposed as an underlying mechanism which is responsible in part for the diffuse vascular injury associated with diabetes.

There is evidence showing that infusion of AGEs into non–diabetic mice results in the generation of TBARS in a variety of organs, including the gingiva. Although TBARS can reflect several different mechanism of oxidants, the inhibitory effect of probucol or N–
acetylcysteine on AGE–induced formation of TBARS points to a central role for reactive oxygen intermediates.\textsuperscript{19} A receptor for AGEs known as RAGE has been identified on the surface of smooth muscle cells, endothelial cells, neurons and monocytes/macrophages. Hyperglycemia results in increased RAGE expression and AGE–RAGE interaction. A group of investigators have suggested the presence of these receptors in the gingiva. Although diabetes is a well-established risk factor for periodontitis, the cellular and molecular basis for this association is unclear. It is likely that generation of reactive oxygen intermediates is linked to AGE production.\textsuperscript{20}

It is recognized that diabetes is associated with more severe periodontal disease, however the level of diabetic control as reflected in glycated hemoglobin (HbA1c) levels, does not correlate well with disease severity. AGE accumulation is greatly increased in many diabetic patients but with heterogeneity. While hyperglycemia is distinctly linked to the onset and progression of diabetic complications, there are many poorly controlled diabetic patients who do not develop significant complications. Conversely, some patients with well-controlled diabetes still develop complications. It is postulated that the differences between individuals in AGE accumulation may explain some of this variable complications within diabetic patients. Our study also indicated that AGE accumulation in gingival tissue can be correlated to periodontal severity and this can be identified as a risk factor for periodontal disease.

The formation of AGES occurs in both central and peripheral diabetic arteries and is thought to contribute greatly to macrovascular complications of diabetes. At the cellular level, AGES have significant effects. Accumulation of AGES not only affects extra cellular matrix components but can affect matrix–to–matrix interactions and cell–to–matrix interactions. The effect on the endothelial cells is an increase in vascular permeability and thrombus formation. The AGE–RAGE interaction on smooth muscle cells results in cellular proliferation within the arterial wall. As AGES are chemotactic for monocytes, AGE–RAGE interaction induces increased cellular oxidant stress and activates the transcription factor NF–KB on monocytes. This then alters the phenotype of the monocyte/macrophage and results in increased production of proinflammatory cytokines and growth factors such as interleukin–7, tumor necrosis factor, platelet–derived growth factor, and insulin–like growth factor. All these cytokines and growth factors have been shown to contribute to the chronic inflammatory process in the formation of atheromatous lesions. Interestingly, oxidant LDL, elevated in many diabetic patients, also activates NF–KB and may result in a similar process. Thus, alteration in lipid and protein metabolism induced by the sustained hyperglycemia characteristic of diabetes may play a major role and provide a common link between all the classic complications of this disease such as periodontitis. These events could underlie, at least in part, the accelerated periodontal disease observed in patients with diabetes. Coupled with diminished protective antioxidant mechanisms in diabetic patients a substantial, biologically relevant oxidant stress is likely to be imparted by AGES to diabetic tissue.\textsuperscript{21,22,23}

Our study has shown that increased breakdown of periodontal structures occurs in diabetic patients, probably resulting from oxidant stress and formation of irreversible glycation of proteins and lipids in diabetic periodontal tissues. However, the small number of patients and different types of diabetes were the limitation of these studies.

\textbf{REFERENCES}


