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Research paper

Metabolic control and periodontal treatment decreases elevated oxidative stress in the early phases of type 1 diabetes onset

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ABSTRACT

Objective: Recently, increasing concern has been focused on the contribution of oxidative stress in the pathology of periodontal disease and diabetes mellitus. Firstly, the present study aimed to analyze gingival crevicular fluid (GCF), salivary, and serum oxidative status in children with type 1 diabetes mellitus (T1DM) at diagnosis and systemically healthy children with and without gingivitis. Additionally, the diabetic patients were reevaluated after diabetes and periodontal treatment.

Design: The study groups were composed of 32 T1DM patients at diagnosis, and age- and gender-matched thirty-six systemically healthy children with (G) and without (H) gingivitis. The diabetic patients who took insulin therapy (1.5 units/kg/day totally) and periodontal treatment (oral hygiene education with professional scaling) were reevaluated after 3 months. The levels of total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were recorded.

Results: GCF, salivary, and serum OSI were elevated in group T1DM compared to the other groups at baseline ($p < 0.05$), and decreased in group T1DM at reevaluation compared to baseline ($p < 0.05$). GCF OSI was positively correlated with periodontal clinical parameters ($p < 0.05$). Glycated hemoglobin was positively correlated with GCF TOS ($r = 0.302$, $p = 0.007$), GCF OSI ($r = 0.346$, $p = 0.002$), salivary TOS ($r = 0.326$, $p = 0.046$), and serum TOS ($r = 0.239$, $p = 0.044$).

Conclusion: The instability in the oxidative status that accompanies diabetes may be considered a significant pathogenic factor of diabetes-related periodontal inflammation.

1. Introduction

Diabetes mellitus is the most prevalent endocrine disease, which affects almost 170 million individuals worldwide and is predicted to increase dramatically in the future (Whiting, Guariguata, Weil, & Shaw, 2011). The incidence of undiagnosed diabetes and impaired glycemic control raises further concerns. Type 1 diabetes mellitus (T1DM), a metabolic disorder resulting from the destruction of insulin-producing pancreatic β-cells that results in insulin loss, represents an extremely significant health problem since it is diagnosed early in life and plays a role in the etiopathogenesis of long-term complications (Bonner-Weir, 2000).

Gingivitis is the most prevalent inflammatory periodontal disease, which can be treated with simple interventions, such as scaling and oral hygiene education; however, if left untreated, it progresses to periodontitis, which involves permanent loss of periodontal tissues, including alveolar bone. Gingivitis is a common disease during childhood and adolescence, and is also a critical complication of T1DM. It has been shown that the prevalence of gingivitis among T1DM patients is nearly twice as high as in non-diabetic individuals (Ryan, Carnu, & Kamer, 2003).

Reactive oxygen species (ROS) are generated by most cells at low amounts in the healthy physiological state (Chapple, 1996). Several antioxidant host-defense systems neutralize these highly toxic molecules, which damage biological molecules, including proteins, lipids, and DNA, significantly impairing cell integrity (Chapple, 1996). As 1–3 billion ROS are generated in a cell daily, the antioxidant defense mechanisms to maintain cell life are crucial (Ames, Shigenaga, & Hagen, 1993).

Hyperglycemia in diabetes impairs ROS scavenging activity, which has been linked to decreased function of scavengers. The balance between oxidants and antioxidants shifts toward oxidative stress in chronic hyperglycemia, leading to tissue damage (Marra et al., 2002). In addition, oxidative stress has been shown to play a role in the

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pathology of diabetes onset and subsequent diabetic complications that represent the main causes of mortality and morbidity in these patients (Karunakaran & Park, 2013). Furthermore, oxidative stress has been reported to increase gradually during the course of diabetes (Firoozrai, Nourbakhsh, & Razzaghy-Azar, 2007), and may damage pancreatic β-cells, causing insulin resistance and decreased insulin production (Karunakaran & Park, 2013).

Increased ROS production that results in oxidative stress is also evident in the periodontal tissues of patients with type 2 diabetes mellitus (Allen et al., 2011). Thus, oxidative stress has been implicated in the development of diabetes-related periodontal complications and the mechanisms that underpin the links between these two diseases (Chapple & Genco, 2013).

To our knowledge, there are no previous studies reporting gingival crevicular fluid (GCF), salivary, and serum total antioxidant status (TAS), total oxidant status (TOS), and the oxidative stress index (OSI) in the early phases of T1DM with regard to periodontal health and disease. Thus, the pathogenesis of periodontal status/diabetes interactions at the onset of T1DM and after achieving metabolic control of diabetes requires further evaluation. Therefore, the aim of the present study was to analyze the role of oxidative stress in children with newly diagnosed T1DM and in systemically healthy controls with and without gingivitis. In addition, the aim of the present study included reevaluation of the diabetic patients after 3 months to investigate the effects of metabolic control and periodontal treatment in the early phases of T1DM onset.

2. Materials and methods

2.1. Study population

This study was approved by the local ethics committee (B.30.2.ŞFÜ.00.50.500\83), and was performed in accordance with the Declaration of Helsinki. The study was performed from May 2014 to August 2015. A total of 68 patients aged 10–16 years were divided into groups as follows: group H (18 systemically and periodontally healthy children; 9 male, 9 female; 11.17 ± 0.83 years), group G (18 systemically healthy children with gingivitis; 10 male, 8 female; 10.40 ± 0.90 years), group T1DM (32 patients at the time of diagnosis of T1DM; 17 male, 15 female; 11.04 ± 2.46 years).

The diabetic children were selected from among individuals treated at Behçet Uz Children's Hospital. The systemically healthy individuals were selected from among patients seeking dental treatment at the Departments of Periodontology and Pediatric Dentistry at Sifa University.

2.2. Clinical evaluation

After obtaining written informed consent from the children's parents, a detailed medical and dental history was taken from each participant. Subjects were excluded if they were obese or if they had syndromes or systemic diseases other than T1DM, a history of ongoing or previous orthodontic and/or periodontal therapy, or a history of taking anti-inflammatories, immunosuppressants, or antibiotics within the previous 3 months. Subjects meeting the inclusion criteria were not on any medications that could affect the manifestation of periodontal disease, such as phenytoin, cyclosporine, or systemic corticosteroids.

2.3. Insulin therapy

The diagnosis and treatment of T1DM was carried out in accordance with the American Diabetes Association criteria (Genuth et al., 2003). All subjects diagnosed with T1DM underwent standard insulin therapy (Chamberlain, Rhinehart, Shaefer, & Neuman, 2016). The total daily insulin dose (1.5 units/kg/day) was consisted of a single dose of insulin glargine (40% of total daily insulin) and insulin lispro (60% of total daily insulin) divided into 3 doses.

2.4. Periodontal examination and treatment

The periodontal examination of each subject was performed by a single examiner (CAA) using a periodontal probe (Goldman-Fox, Hu-Friedy, Chicago, IL, USA). The periodontal clinical parameters were as follows: the simplified-oral hygiene index divided by debris (DI-S) and calculus (CI-S) indices (Suomi & Doyle, 1972), and the gingival bleeding index (GBI) (Goncalves et al., 2008). All indices were assessed for the six Ramfjord teeth, that is, on each permanent tooth other than deciduous or erupting teeth. No participating individuals had sites with a probing depth of >3 mm or a clinical attachment loss of >1 mm.

The periodontal diagnosis was made according to the radiographic and clinical criteria suggested by the American Academy of Periodontology (Armitage, 1999). Periapical and/or bitewing radiographs were taken to evaluate the alveolar bone. All diabetic subjects received detailed oral hygiene education with professional scaling during the hospitalization period, which lasted 4 to 7 days.

2.5. GCF sampling

The areas for the GCF samples were isolated with cotton rolls, and GCF samples were taken after the removal of supragingival plaque with sterile periodontal instruments. For each of the subjects, GCF samples were obtained from the six Ramfjord teeth, and the samples were collected 30 s after gentle air-drying by placing paper strips (Periopaper, Oraflow, Smithtown, NY, USA) in the gingival crevices. Samples with gingival bleeding were excluded. The strips were placed in polypropylene tubes and stored at −80 °C.

2.6. Saliva sampling

The participants were asked to avoid eating or drinking for one hour before the sample collection. Unstimulated whole-saliva samples were collected by expectorating into 2-ml polypropylene tubes before clinical periodontal measurements. Saliva samples were centrifuged at 800 × *g* for 2 min. The resultant supernatants of the samples were stored at −80 °C for further analysis.

2.7. Serum sampling and biochemical analysis

Blood samples were drawn by using a standard venipuncture method. The serum was separated after centrifugation performed at 3000*g* for 10 min, and was then placed in polypropylene tubes. Serum biochemical analyses were performed on fresh samples. The sample aliquots were immediately frozen and stored at −80 °C.

2.8. TAS, TOS, and OSI assays

GCF was recovered from the paper strips by gently vortexing the strips in 250 μL of filtered phosphate-buffered saline (pH 7.4). TOS and TAS levels were measured using commercially available assay kits (Rel Assay, Mega Tıp, Gaziantep, Turkey).

The TAS assay determined the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization reduced by antioxidants in the sample. The change in absorbance indicated the level of total antioxidants present in the sample. The assay was calibrated with a stable antioxidant standard solution, Trolox equivalent, which is a vitamin E analog. Thus, the results were expressed as mmol of Trolox equivalents per liter (Erel, 2004).

The TOS assay determined the oxidation reaction of the ferrous ion-chelator complex to ferric ion, which produces a colored complex by the oxidants in the sample. The color intensity was measured by spectrophotometric analysis, which indicated the total amount of oxidant molecules. The assay results, calibrated by H_2O_2, were expressed in terms of μmol of H_2O_2 equivalents per liter (Erel, 2005).

As an indicator of the degree of oxidative stress, the total H_2O_2-to-