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Clinical Paper
Dental Implants

Impact of type 2 diabetes on the gene expression of bone-related factors at sites receiving dental implants

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Abstract. This study evaluated the influence of type 2 diabetes mellitus (T2DM) on the gene expression of bone-related factors in alveolar bone tissue from sites designated to receive dental implants. Bone biopsies were harvested from sites of planned implants for 19 systemically healthy patients and 35 patients with T2DM (17 with better-controlled T2DM (glycated haemoglobin (HbA1c) levels $\leq$8%) and 18 with poorly controlled T2DM (HbA1c levels >8%)). The mRNA levels of tumour necrosis factor alpha, transforming growth factor beta, receptor activator of the nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), runt-related transcription factor 2, alkaline phosphatase, bone sialoprotein (BSP), type I collagen (COL-I), and osteocalcin were evaluated by quantitative real-time polymerase chain reaction. T2DM up-regulates RANKL levels and the ratio of RANKL/OPG, whereas it down-regulates COL-I and BSP expression ($P < 0.05$). Higher mRNA levels of RANKL/OPG were observed in the poorly controlled T2DM patients compared to those with better-controlled T2DM and systemically healthy patients ($P < 0.05$). A lower amount of COL-I and BSP was detected in the biopsies from individuals with poorly controlled T2DM compared to systemically healthy patients ($P < 0.05$). In conclusion, RANKL, RANKL/OPG, COL-I, and BSP are negatively affected in diabetics. Additionally, the patient's glycaemic status appears to modulate bone-related genes in a different manner.

Key words: dental implants; biological markers; gene expression; real-time polymerase chain reaction; bone and bones; diabetes mellitus type 2.

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Type 2 diabetes mellitus (T2DM) is a systemic disease with several complications affecting the quality and length of life. The high prevalence of periodontitis and subsequent tooth loss in diabetics represents an important health implication,[1] and treatment with dental implants is considered a therapeutic strategy for replacing lost teeth in these patients. This treatment improves masticatory function and dietary intake, which are essential in the management and improvement of

quality of life of T2DM patients. Although dental implant therapy has been described as safe and has a high success rate, there are certain conditions that can interfere in the predictability of outcomes.[2]

In this context, hyperglycaemia has been linked to an increased risk of T2DM co-morbidities, including reduced bone turnover and impaired bone mineralization and density.[3,4] Indeed, the poor glycaemic control in diabetic patients appears to negatively interfere with bone repair around implants and compromises implant stability during the healing period.[5–7] However, the precise cellular and molecular mechanisms that may lead to the greater impairment in bone repair of T2DM patients when compared to patients without diabetes, remain unknown.

The aim of this study was to evaluate the gene expression of biomarkers of bone turnover in alveolar bone biopsies from systemically healthy patients compared to individuals with T2DM, both better-controlled T2DM and poorly controlled T2DM. The following biomarkers were assessed: tumour necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), receptor activator of the nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), runt-related transcription factor 2 (Runx2), alkaline phosphatase (ALP), bone sialoprotein (BSP), type I collagen (COL-I), and osteocalcin (OC). These molecules are key markers in the skeleton and immune systems, and are thought to be critical in bone tissue. The hypothesis of this study was that T2DM and glycaemic control could modulate the expression of these bone-related genes in the alveolar bone of sites designated to receive dental implants.

Materials and methods

Patient population

Thirty-five patients with T2DM and 19 patients without diabetes (total 54 patients) were selected; 25 were male and 29 were female, and they ranged in age from 37 to 70 years. All individuals were recruited from patients referred to Paulista University between April 2012 and February 2013. Detailed medical records were obtained and patients who fulfilled the inclusion/exclusion criteria (outlined below) were invited to participate in the study. All eligible patients were thoroughly informed of the nature of the study and the potential risks and benefits of their participation, and they each signed an informed consent document. This study was approved by the university ethics committee.

Inclusion and exclusion criteria

To be included in this study, all patients were required to be aged between 35 and 70 years and have a posterior mandibular single-tooth edentulous area indicated for rehabilitation with a dental implant; the extraction had to have occurred at least 12 months before treatment. The diabetic subjects had to have T2DM, diagnosed by a physician, with a duration of at least 5 years. Individuals were under a diet regimen and/or the use of oral hypoglycaemic agents (metformin or glibenclamide).

The exclusion criteria were pregnancy, lactation, smoking or ex-smokers, other systemic conditions that could affect bone metabolism (e.g., immunological disorders), the use of anti-inflammatory and immunosuppressive medications, patients who required bone grafts before or alongside the implant surgery, and a history of previous regenerative procedures in the area designated for implant therapy. Patients with major complications associated with T2DM, i.e. cardiovascular and peripheral vascular diseases (ulcers, gangrene, or amputation), neuropathy, and nephropathy, were also excluded.

Fasting plasma glucose and glycated haemoglobin monitoring

A single laboratory (Clinical Analysis Laboratory, Paulista University) executed all blood analyses, including fasting plasma glucose (FPG) and glycated haemoglobin (HbA1c) monitoring. FPG was measured using the glucose oxidase method and HbA1c was measured by high-performance liquid chromatography. Patients who had HbA1c values >8% were considered to have poorly controlled T2DM, whereas patients who presented HbA1c levels ≤8% were classified as having better-controlled T2DM.[8]

Experimental groups

The patients were divided into systemically healthy patients without diabetes ($n = 19$) and patients with T2DM ($n = 35$). Based on their glycaemic status, diabetic patients were further subdivided into those with better-controlled T2DM ($n = 17$; HbA1c levels ≤8%) and poorly controlled T2DM ($n = 18$; HbA1c levels >8%).

Implant therapy and sample collection

All surgeries were performed by the same operator (AC) and all patients received a single-stage dental implant. The surgical area was anesthetized and mucoperiosteal incisions were made in the alveolar ridge mucosa. Before implant placement, a bone tissue biopsy was collected from the site designated to receive the dental implant, using a device for collecting bone material during implant site preparation. The device includes a container, which collects the bone that is removed from the patient during the surgical procedure through a suction hose. The samples collected were later stored in tubes containing RNA*later* for RNA stabilization (Life Technologies, Rockville, MD, USA) and stored at −70 °C until subsequent analysis.

The surgical sequence followed the protocol described by the implant company (S.I.N. Implante, São Paulo, Brazil). Amoxicillin (2 g/h before the procedure) and postoperative sodium-dipyrone (500 mg every 6 h for 2 days), as well as 0.12% chlorhexidine mouthwash (every 12 h for 7 days) were indicated. The placement of the prosthesis was performed within a period of 4 months.

Gene expression analysis

For the gene expression analysis, the mRNA levels of TNF-α, TGF-β, RANKL, OPG, Runx2, ALP, BSP, COL-I, and OC were determined. Total RNA was isolated from the biopsies by the TRIzol method (Gibco BRL, Life Technologies). RNA samples were re-suspended in diethylpyrocarbonate-treated water and stored at −70 °C. The RNA concentration was determined using a spectrophotometer (NanoDrop 1000; NanoDrop Technologies LLC, Wilmington, DE, USA).

Total RNA was DNase-treated (Ambion TURBO DNA-free Kit; Life Technologies) and 1 μg was used for complementary DNA (cDNA) synthesis. The reaction was carried out using the First-Strand cDNA Synthesis Kit (Roche Diagnostics, Indianapolis, IN, USA). Primers were designed using probe design software (LightCycler Probe Design Software; Roche Diagnostics GmbH, Mannheim, Germany) (Table 1). The real-time quantitative polymerase chain reaction (qPCR) (LightCycler System; Roche Diagnostics GmbH) was performed using a SYBR Green kit (FastStart DNA Masterplus SYBR Green; Roche Diagnostics, Indianapolis, IN, USA). The results were expressed as the relative amounts of the target gene using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the inner reference gene, by means of the relative quantification tool (LightCycler Software 4; Roche Diagnostics GmbH).