Blood Profile and Histology in Oral Infections Associated with Diabetes

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Abstract

Introduction: We aimed to investigate the relationship between blood profile and histologic findings in both apical periodontitis (AP) and periodontal disease (PD) associated with diabetes. **Methods:** Wistar rats (N = 80) were assigned to the following 8 groups: control, AP, PD, AP associated with PD, diabetes, diabetes with AP, diabetes with PD, and diabetes with AP and PD. Diabetes mellitus (DM) was induced with streptozotocin, AP was induced by exposure to the oral environment, and PD was induced using periodontal ligature. After 30 days, blood samples were collected, and the rats were euthanized. Subsequently, the maxillae were processed for light microscopy. Hematologic examinations were conducted to determine the total number of erythrocytes and leukocytes, erythrocyte constant, and blood glucose level. One-way analysis of variance and Kruskal-Wallis tests were used for statistical analysis, and the significance was set at $P < .05$. **Results:** A significant correlation was found between the histologic findings and blood parameters. **Conclusions:** In conclusion, diabetes accelerated the development and progression of AP and PD in the rats and caused an increase in the average erythrocyte volume as well as the leukocyte and neutrophil counts. Oral infections increase the total number of leukocytes, the number of neutrophils and lymphocytes, and blood glucose concentrations in DM rats. *(J Endod 2014;40:1139–1144)*

Key Words

Apical periodontitis, blood count, diabetes, oral infections, periodontal disease

Recent studies have emphasized the probability of a relationship between inflammatory disorders of the oral cavity and pathologic conditions in several organs and systems (1–10). In this regard, acute and chronic apical periodontitis (AP) may play a major role (1, 8–10). Similarly, it is hypothesized that systemic conditions, such as cardiovascular disease (1), renal disease (2), cerebral abscesses (3), pulmonary disease (4), osteoporosis (5), and diabetes mellitus (DM), (6–10) are possibly associated with oral infections.

DM is a systemic disease characterized either by a disturbance in insulin production or insulin resistance in target tissues, which results in hyperglycemia. In 2011, 366 million people were reported to have DM, and this number is expected to increase to 552 million by 2030 (11). If uncontrolled, DM leads to health complications, increasing the susceptibility to infection and inflammation, such as AP and periodontal disease (PD) (6–10).

The relationship between DM and oral diseases has been studied extensively in the past (10). Patients with DM show an increasing severity of chronic periodontitis and periodontal abscesses and an increasing predisposition to infection (6). PD is 1 of the most prevalent complications in diabetic patients (12), in addition to AP and endodontic infection (13). In fact, AP and PD are very common oral infections worldwide and have similar incidences (14).

However, the possible aggravating effect of PD on the diabetic condition has not been investigated sufficiently (6). Studies performed on diabetic rats show that PD may contribute to insulin intolerance in subjects (7), and these findings are consistent with the findings of some human studies.

Some studies report that endodontic infections can be more aggressive and destructive in patients with DM (13) because uncontrolled DM may result in poor healing (15). Rats with DM are seemingly more affected by endodontic problems than normal rats and show larger periapical lesions (16). In rats, type 2 DM results in a metabolic condition that predisposes the animal to enhanced development of chronic lesions (16). Furthermore, DM may modify healing (15), structural components (17) and parameters of the antioxidant system (18), and dental pulp tissue and may increase bacterial levels in root canals (19). Additionally, patients with DM have impaired host defense mechanisms (20). It is for these reasons that DM can easily lead to necrosis and the establishment of AP (10). Despite this information, the progression of such lesions and their association with and impact on systemic conditions have not been extensively studied, especially regarding lesions associated with PD.

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One method to evaluate the association of oral infections with diabetes and its effect on health status and body homeostasis is to perform a hematologic analysis (21). Blood tests are a very useful diagnostic tool. Blood is composed of several different cells, salts, and proteins. A quantitative and qualitative hematologic evaluation may facilitate disease diagnosis and serve as a useful indicator of response to inflammation and infection, thereby allowing assessment of the physiological status of populations (21). Therefore, this study was developed in part because of an insufficient amount of research regarding the hematologic and local inflammatory response to oral infections in subjects with diabetes.

Materials and Methods

Experimental Animals

The experimental protocol was approved by the Institutional Ethics Committee (CEUA 539-2012) of Universidade Estadual Paulista, São Paulo, Brazil, and conducted in accordance with relevant guidelines. Eighty male Wistar rats (*Rattus norvegicus albinus*), each weighing 200–250 g, were used in this study. The animals were housed in temperature-controlled rooms and given ad libitum access to water and food.

Induction of DM

The Wistar rats were divided into the following 8 groups comprising 10 rats each: control (C), AP, PD, apical periodontitis associated with periodontal disease (AP + PD), DM, DM with AP (DM + AP), DM with PD (DM + PD), and DM with AP and PD (DM + AP + PD).

The rats were then administered anesthesia via intramuscular injections of ketamine (87 mg/kg, Francotar; Virbac do Brasil Ind e Com Ltda; Roseira, SP, Brazil]) and xylazine (13 mg/kg, Rompum; Bayer SA, São Paulo, SP, Brazil). The subjects were randomly assigned to receive injections in the penile vein; the injections contained either a citrate buffer solution 0.01 mol/L with a pH of 4.5 (C, AP, PD, and AP + PD groups, $n = 40$) or streptozotocin (Sigma-Aldrich Corp, St Louis, MO) (DM, DM + AP, DM + PD, and DM + AP + PD groups, $n = 40$) dissolved in a citrate buffer solution at 35 mg/kg body weight for the experimental induction of diabetes (11).

After 1 week, rats with a blood glucose level of more than 200 mg/dL were used in the DM groups (9, 22). Once hyperglycemia was confirmed, all the subjects were sedated for the induction of oral infections.

Induction of Oral Infections

To induce AP, surgical round burs were used to expose the pulp on the mesial surfaces of right upper first molars (Broca Ln Long Neck; Maillefer, Dentsply Ind e Com Ltda, Petrópolis, Brazil) (AP, AP + PD, DM + AP, and DM + AP + PD groups) (9, 16). To induce PD, sterile silk ligatures (Ethicon, Johnson and Johnson, São Paulo, Brazil) were tied around the maxillary left second molars (PD, AP + PD, DM + PD, and DM + AP + PD groups) (9).

Blood Sample Collection and Determination of Hematologic Parameters

At 30 days, the subjects were again anesthetized with the same protocol as previously described, and a cardiac puncture was performed to collect 5 mL blood from each of the subjects. The samples were placed in EDTA, homogenized, and immediately transferred to a technician, who was blinded to the case status, for processing.

The following parameters were analyzed using an automatic analyzer (ABX Micros ABC Vet; Horiba ABX Diagnostics, Montpellier, France): red blood cell concentration; packed cell volume; mean corpuscular hemoglobin level; mean corpuscular volume (MCV); mean corpuscular hemoglobin concentration; and leukocyte, neutrophil, lymphocyte, monocyte, basophil, and eosinophil counts. Next, the results of each of these hematologic parameters were analyzed by analysis of variance followed by the Tukey test ($P < .05$).

Tissue Processing and Morphometric Evaluation

After blood sample collection, the subjects were killed by an overdose of anesthetic solution. Their maxillae were removed, postfixed in neutral buffered formalin for 48 hours, and then decalcified in buffered (pH = 8) 17% EDTA (Sigma Chemical Co, St Louis, MO). Subsequently, they were rinsed again with sterile water before being dehydrated with ethanol, cleared in xylene, and finally embedded in paraffin. Serial slices (6 μm) were prepared in the mesiodistal plane and stained with hematoxylin-eosin. The slices were examined sequentially under an optical microscope (DM 4000 B; Leica, Wetzlar, Germany).

Inflammatory infiltrate was evaluated for its intensity and extension. The average number of cells per field was noted as well as whether the inflammation extended beyond the apical foramen (AP groups) or the cementoenamel junction (PD groups). For each experimental group, the number of cells was calculated as the average of 10 separate areas ($\times$400 magnification) (22).

The intensity of the inflammatory infiltrate was graded as follows: absent (0 to few inflammatory cells: score 1), mild (<25 inflammatory cells: score 2), moderate (25–125 inflammatory cells: score 3), or severe (>125 inflammatory cells: score 4). A 0.5-mm square area of the periapical portion of the mesial root was examined, and the cell count per unit area was calculated. A single calibrated operator, who was blind to the specimen group, performed the statistical analysis of the results (Mann-Whitney U test, $P < .05$) between the AP and DM + AP groups and the PD and DM + PD groups.

For each rat subject, 5 serial histologic sections were measured histometrically using an image processing system, which consisted of a light microscope (DM 4000 B, Leica), a color camera (DFC 500, Leica), a color image processor (Leica Qwin V3 software, Leica), and a personal computer (Intel Pentium 4, 2.80 GHZ, Windows XP SP3). For the AP and DM + AP groups, the area of the periapical lesion was histometrically measured.

Alveolar bone loss was evaluated by measuring the distance between the cementoenamel junction and the buccal alveolar bone crest in 10 separate areas, each from the side of the maxilla, for the CG, PD, DM, and DM + PD groups. The amount of bone loss and the periodontal ligament were measured 3 times in each section by the same examiner (to reduce data variation) on different days (22).

The length and area of the maxilla were determined for each side, and the average value (mean $\pm$ standard deviation) was calculated for each experimental group. A 1-way analysis of variance along with either the Tukey test or the Student t test ($P < .05$) were performed to compare the length and area data between oral infection groups with or without DM. All statistical tests were performed using Pacotico software (Visual FoxPro; Microsoft, Redmond, WA).

Results

Blood Profile

Blood counts were performed for all the subjects 30 days postoperatively. The effect of streptozotocin-induced DM on the hematologic parameters of subjects with oral infections was investigated and then compared with the corresponding findings of normal subjects without DM. The results of the complete blood count and differential white blood cell count are shown in Table 1.