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Mission of Mercy, Southern MD	October 2011

Abstract

Title of Thesis: The Relationship Between Short Term Healing of Periapical Lesions and Glycemic Control: A Pilot Study

Zuwu Zhou, Master of Science 2019

Thesis Directed by: Dr. Priya Chand, BDS MS (Director of ASE Endodontics Residency Program)

The aim of this study was to evaluate the relationship between HbA1c level and short-term healing of periapical lesions after non-surgical root canal treatment (NSRCT). After NSRCT, digital periapical radiographs were taken and blood samples were drawn for HbA1c analysis. To assess short-term healing (PAI difference), subjects returned after six months for a radiograph and blood draw. A calibration exercise established the reliability of the PAI scoring process. Thirty-eight subjects returned for the recall. The relationship between HbA1c level and also the co-variables, with healing was explained using Spearman's rho and logistic regression. There was a significant correlation between healing and HbA1c level (r_s = -.52, $p \leq .0001$), age (r_s = -.44, $p \leq .003$), recall days (r_s =.29, $p \leq .036$), and cardiovascular status (r_s = -.34, $p \leq .018$). A final logistic regression showed a significant relationship between HbA1c level and short-term healing of periapical lesions (R = .62, $p \leq .05$).

The Relationship Between Short Term Healing of Periapical Lesions and Glycemic Control: A Pilot Study

by
Zuwu Zhou

Thesis submitted to the Faculty of the Graduate School
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Coronary Heart Disease (CHD)

Acute Coronary Syndrome (ACS)

Glycolated hemoglobin (HbA1c)

The Periapical Index (PAI)

Advanced Specialty Education in Endodontics Program at the University of Maryland

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Introduction

Diabetes is a chronic illness characterized by high levels of blood glucose resulting from inadequate production of insulin, utilization of insulin or both. Insulin is secreted by the pancreas, absorbed in muscle, liver, and adipose tissues for glycogen and fatty-acid synthesis (1).

More than 30 million Americans live with diabetes and are susceptible to developing micro and macrovascular diseases such as kidney failure, blindness, cardiovascular disease, and even premature death (2, 3). The most common forms of diabetes are Type 1 and Type 2 (3). Type 1 diabetes is common in children, and these individuals are often unable to produce insulin as result of an autoimmune process that destroys insulin-secreting beta cells of the pancreatic islets (2, 3). Type 2 diabetes most affects adults and is characterized by insulin resistance or insufficient production of insulin (3). More than 90% of patients are diagnosed with Type 2 diabetes (2).

While 30 million Americans are living with diabetes, over 90 million are living with prediabetes, a serious health condition that predisposes them to type 2 diabetes and other chronic illnesses (2, 4). In fact, 9 out of 10 people with prediabetes are not aware that they have it (4). Individuals with pre-diabetes show a reduction in insulin sensitivity years before diagnosis of diabetes has been made (5). However, they usually show a compensatory increase in pancreatic B cell activity and insulin secretion several years before conversion to diabetes take place (5). Once the pancreatic B cells have lost their compensatory mechanisms for insulin resistance, glucose concentrations rise and diabetes develops (5) . More than 33% of patients with prediabetes will progress to Type 2 within three to five years, and 70% will develop diabetes during their lifetime (4, 5).

If left untreated individuals are at increased risk of developing comorbidities such as cardiovascular and neurological diseases in addition to infections more frequent and severe leading to higher incidence of bacteria and associated mortality than non-diabetic individuals(6, 7). Diabetes impairs host immune response which increases the incidence of bacteremia and bacteria-related mortality rates. Individuals with diabetes have 1.8x higher chance of bacterial-related mortality due to their altered immune functions (7) such as reduction of polymorphonuclear leukocyte and T-cell functions, leukocyte adherence, chemotaxis, and phagocytosis (8). Antioxidant enzymes responsible for scavenging harmful free radicals are also impaired. Significant reductions in antioxidant actions of superoxide dismutase, glutathione peroxidase, and glutathione reductase have been reported and thus contribute to the development of diabetic complications (9). As a result, diabetic individuals are more susceptible to infections due to reduced immune functions.

Other contributing factors to diabetic complications include the formation of advanced glycation end products (AGE) (10). Hyperglycemia promotes a chemical reaction between sugars and proteins that forms glycated proteins contributing to diabetic complications (10). Glycated proteins are formed when sugars react with amino groups from protein that gives rise to poorly characterized structures called advanced glycation end products (AGE) (10). Advanced glycation end products bind to AGE receptors (RAGE) on smooth muscle cells, macrophages, endothelial cells, and astrocytes. Accumulation of AGEs can trigger activation of pro-inflammatory cytokines, cause delay in wound healing, and narrowing and occlusion of blood vessels leading to cardiovascular complications (10).

Diabetes has a substantial impact on oral health predisposing individuals to caries, pulpal and periapical pathosis, and periodontal disease (11). Hyperglycemia can lead to impaired circulation resulting in changes to the dental pulp and periapical tissues (11, 12). High levels of blood glucose have been shown to inhibit macrophage functions which lead to delayed wounding healing of dental pulp and surrounding periapical tissues(13). Additionally, hyperglycemia is associated with upregulation of osteoclastic cytokines that increase bone resorption (14).

Glycated Hemoglobin (HbA1c)

Glycohemoglobin is formed through a nonenzymatic chemical action covalent bond between glucose and hemoglobin (Hb) (15). Hemoglobin can be broken down into minor components through cation exchange chromatography and be given designations HbA0, HbA1a, HbA1b, and HbA1c according to their order of elution (15). Blood glucose can be measured through fasting blood glucose (FBG), postprandial glucose (PPG), and hemoglobin A1c (HbA1c). The HbA1c test categorizes patients at 3 levels: normal (score of 5.6% or less), pre-diabetic (score between 5.7% to 6.4%), and diabetic (score of 6.5% or higher) (16).

Fasting and postprandial glucose tests only measures blood glucose level at a single moment of the day and maybe inaccurate for chronic diabetic patients (17). HbA1c test, other the other hand, offers several advantages to blood glucose assessment tests. HbA1c is formed from attachment of glucose to hemoglobin and HbA1c test is highly correlated with mean blood glucose level over the past three months (18, 19). HbA1c test does not require patients to fast eight hours prior to testing as some individuals may not be able to abstain from food for eight hours (17). One HbA1c measurement is equivalent to hundreds

of fasting glucose levels and also provides peak levels of postprandial glucose concentration (17). HBA1c reflects longer term glycemic levels and is a more accurate measurement of detecting diabetes than blood glucose assessment tests (20).

HbA1c has less glycemic measurement variability than other blood glucose tests (17). Fasting glucose measurements can differ up to 16.6% in contrast to the low variability of 3.6% in HbA1c tests. Therefore HbA1c provides more reliable clinical information (17). Comparison tests show higher stability over time for hemoglobin-based test than plasma glucose-based test (17). Blood samples in transit to the lab for analysis can lose significant amount of glucose before being processed (17). A decrease of 5 – 7% of glucose concentration occurs each hour that may produce a noticeable preanalytical variability in fasting blood glucose test, but a negligible variability in HbA1c test (17).

While HbA1c has many advantages, there are also some disadvantages. A high HbA1c number represents high glycation of proteins secondary to high blood glucose (17). A delay in diagnosis by up to weeks or months can occur if relying solely on HbA1c because an increase in HbA1c is detected only after an increase in blood glucose. (17). Since the HbA1c test measures the exposure of glucose bound to erythrocytes over a period of the last 90-120 days (21), this test will not detect acute hyperglycemia unlike a two-hour postprandial glucose tolerance test. A high two-hour postprandial glucose tolerance test indicates B-cell function impairment, a significant pathophysiological defect of diabetes which requires proper medical management (17). Furthermore, medical conditions like malaria, chronic anemia, major blood loss, hemolysis, uremia and pregnancy can produce unreliable HbA1c results (17). Any systemic or environmental condition that affects

hemoglobin or erythrocyte could reduce the accuracy of HbA1c test results since the test measures blood glucose bound to red blood cells (17).

Diabetes and Periodontal Disease

The link between diabetes and periodontal disease has been well studied. Many studies report diabetes to be a significant risk factor to progression of periodontal disease. Sznajder et al. (22) evaluated periodontal conditions of diabetic and nodiabetic patients and found those with diabetes showed significantly more attachment loss and gingival inflammation. Both groups had similar plaque and calculus scores indicating similar oral hygiene (22).

Hugoson et al. (23) showed a temporal association between Type 1 diabetes and periodontal disease. Subjects in this study who lived with Type 1 diabetes on average of 29 years had poorer periodontal health than those who had diabetes for 5 years. The longer duration diabetes group had more teeth with ≥6mm probing depths than the control group (23). Significantly more alveolar bone loss was also found in the longer duration diabetes group than the shorter duration diabetic group and the control group. While all subjects with diabetes showed significantly more gingivitis than controls, the longer they had been living with diabetes the more advanced the periodontal disease and the bone loss (23).

Enrich et al. (24) examined the association between Type 2 diabetes and periodontal disease. Results of their study showed Type 2 diabetes increased risk of attachment loss by 2.81-fold and risk of alveolar bone loss by 3.43-fold (24). Their findings supported those by Hugoson et al.

Kaur et al. (25) confirmed the findings by Hugoson et al. and Enrich et al. that both Type 1 and Type 2 diabetes influences the development and progression of periodontal disease. Considering that Type 1 and Type 2 diabetes affect different age groups, this study found significant association between Type 1 DM with attachment loss in the 20-59 year age group, and sigifincant association between Type 2 DM with attachment loss in the 50-81 years age group. In addition to loss of attachement in both diabetes groups, tooth loss was also a significant finding related to diabetes status (25).

While diabetic status affects periodontal health, periodontal health also impacts diabetic control. Grossi et al. (26) evaluated the effect of periodontal treatment on glycemic control in Type 2 DM subjects with periodontal disease. Diabetic status was determined by HbA_{1c} test at base line and at follow-up visits. At the 3-month follow up, subjects who received scaling and root planning (SRP) with systemic doxycycline showed a significant reduction in HbA1c level by as much as 10% (26). This study provided evidence that reduction of periodontal infection improved diabetic control short term. Another study by Sun et al. also found periodontal therapy significantly reduced HbA1c levels within three months in Type 2 DM subjects (27).

A systematic review and meta-analysis by Janket et al. (28) showed SRP had only a small impact on glycemic control. Their meta-analysis showed a 0.38% reduction in HbA1c after periodontal therapy in Type 1 and 2 DM subjects and 0.66% reduction in only those with Type 2 DM (28). The reduction in HbA1c, however, was not significant.

A multicenter clinical study by Engebretson et al. (29) also found no significant reduction of HbA1c levels in patients with type 2 diabetes and chronic periodontal disease.

Patients with Type 2 diabetes and chronic periodontitis were treated with scaling and root planning. Six-month follow up showed a significant reduction in probing depth and clinical attachment loss after SRP with no significant reduction in HbA1c. In fact, an increase of 0.17% and 0.11% was found in treatment and control groups, respectively (29). Findings from Janket and Engebretson suggested successful periodontal therapy did not influence glycemic control in diabetic patients.

Systemic Diseases and Endodontic Disease

An association between systemic diseases and endodontic disease has been reported in the literature, but the level of evidence remains weak. Several studies have reported an association of cardiovascular disease to endodontic disease. A Veterans Affairs (VA) longitudinal study found lesions of endodontic origin contributed to development of coronary heart disease (CHD) in males under 40 years of age (30). A Finnish study showed a correlation between endodontic lesions and acute coronary syndrome (ACS) (31). Patients with two or more untreated endodontic lesion were 131-149% more likely to develop ACS (31).

Wang et al. (32) found a correlation between hypertension, diabetes, and cardiovascular disease (CVD) and tooth extraction after non-surgical root canal treatment (NSRCT). In this prospective study patients with hypertension, coronary artery disease, or diabetes were 1.75, 1.70, and 1.79 times more likely to have their root canal treated teeth extracted (32). However, the authors did not specify whether the teeth were extracted due to endodontic or restorative failure.

Sickle cell anemia is also associated with endodontic disease. Sickle cell anemia (SCA) is a genetic disorder which results in production of abnormally shaped hemoglobin production which can lead to vascular obstruction (33). Patients with SCA are 8.33 times more likely to develop pulpal necrosis due to occlusion of pulp vasculature (33). Viral infections have also been identified to be associated with endodontic disease. Human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) have been detected in periapical lesions in symptomatic teeth (34).

In addition to seeing an association between systemic diseases and endodontic disease, a relationship between smoking and endodontic disease also exists. Tabaco smoke affects immune response by interfering with neutrophil migration and function (35). Duncan et al. showed more endodontically treated teeth are lost than untreated teeth in smokers (35). Segura-Egea et al. (36) found a significantly higher prevalence of apical periodontitis in smokers. A significant difference in percentage of endodontically treated teeth was also found between smokers and non-smokers, with smokers associated with more root canal treated teeth (36).

Hyperglycemia and Endodontic Pathosis in Animals

Several studies examined the association between hyperglycemia and pulpal and periapical inflammation. Iwama et al. (37) investigated the effects of Type 2 DM on the development of apical periodontitis in teeth with pulpal exposures. Twenty normal rats and twenty Type 2 DM rats were equally divided into four groups: normal rats (A and B), and Type 2 DM rats (C and D). Rats in groups A and C were fed normal diet with tap water, and rats in groups B and D were given normal diet with 30% sucrose solution. The rats were anesthetized and pulp chamber of mandibular first molar was exposed with surgical

bur to the oral cavity. After the rats were sacrificed, histological analysis showed diabetic rats fed with 30% sucrose containing diet showed more pulpal necrosis, alveolar bone resorption, and development of abscesses. The diabetic rats fed with 30% sucrose solution also showed significantly larger areas of periapical lesions, which suggested that diabetes modified metabolic conditions which enhanced the development of periapical lesions (37).

Garber et al. (38) evaluated pulpal healing in rats with elevated blood glucose. In this study, one group of rats received streptozotocin injection to induce hyperglycemia whereas the control group received saline injection. Pulp exposures on maxillary first molars were induced with surgical burs that were subsequently restored with mineral trioxide aggregate (MTA), a biocompatible material capable of inducing dentin bridge formation (38, 39). The rats were sacrificed and histological analysis revealed significantly less dentin bridge formation and more pulpal inflammation in diabetes induced rats (38). This study showed hyperglycemia was associated with increased pulpal inflammation and reduced capacity for pulpal tissue repair.

Hyperglycemia and Endodontic Pathosis in Humans

Hyperglycemia or high blood glucose concentration is a defining feature of diabetes and has been shown to alter immune response leading to increased susceptibility to infection (40). While studies on diabetes and periodontal disease are abundant in the literature, studies on diabetes relating to endodontic disease are fewer in comparison. Several studies have shown reduced endodontic success in patients with diabetes and thus understanding the role hyperglycemic plays in endodontics is an important step in achieving better outcomes.

Cheraskin et al. (41) conducted a classic study on the effect of glucose metabolism on the healing of endodontic lesions after non-surgical root canal treatment (NSRCT). Twenty-five patients with teeth showing periapical radiolucencies (PARL) requiring non-surgical endodontic therapy were enrolled in the study. Two hour postprandial blood glucose was used to assess the subjects' glucose metabolism. Subjects were divided into two groups, lower and higher blood glucose levels and were followed over 30 weeks. At the end of the study, 74% of healthy subjects had a reduction in the size of periapical lesions compared to only 48% in those with diabetes (41). This classic study showed the association between blood glucose control and the healing of endodontic lesions after NSRCT.

Falk et al. (42) conducted a cross sectional study which examined the relationship between Type 1 DM and endodontic healing after NSRCT. No significant difference in prevalence of periapical lesions between subjects with diabetes and controls was found. However, women living with Type 1 DM longer had significantly higher percentage of endodontically treated teeth with residual periapical lesions than women with recent diagnoses of Type 1 DM or healthy controls. No significant difference was found in men (42). This was the first study to report the impact of duration of diabetes on healing after NSRCT.

Britto et al. (43) retrospectively examined patients with Type 1 or 2 DM and prevalence of apical periodontitis in root canal treated and non-treated teeth. This study found men with Type 2 DM had higher prevalence of apical periodontitis and were more likely to have residual lesions after endodontic treatment (43). In the same year, Fouad and Burleson (44) examined NSRCT success in patients with and without diabetes. They found

no significant difference in successful and failed treatments between patients with diabetes and those in cases without pre-operative periapical radiolucencies. In the presence of pre-operative periapical radiolucencies, subjects with diabetes had significantly higher root canal treatment failures than healthy subjects. They also found periodontal disease did not significantly impact endodontic outcome (44).

All previous endodontic studies relied on patients' self reported diabetes status or post prandial glucose to determine patients' diabetes status. Sanchez-Dominguez et al. (45) used the HbA1c test to evaluate the prevalence of apical periodontitis in endodontically treated teeth in patients with Type 2 DM. Subjects with diabetes were divided into two groups based on their level of glycemic control: a well-controlled group (HbA1c <6.5%) and a poor controlled group (HbA1c >6.5%). There was a significant association between periapical status of endodontically treated teeth and HbA1c level. Subjects with HbA1c >6.5% tend to have a higher prevalence of apical periodontitis than those in the well-controlled group (45).

While previous studies showed an increased prevalence of apical periodontitis in root canal treated teeth in subjects with diabetes, Marotta et al. did not find any difference (41-46). They found the prevalence of apical periodontitis was significantly higher in non-root canal treated teeth in subjects with Type 2 DM, but not in previously endodontically treated teeth (46). Their findings suggest diabetes modifies the immune system which predisposes the host to apical disease but did not affect healing after root canal treatment (46).

A recent longitudinal study by Arya et al. (47) examined the healing of apical periodontitis after NSRCT in subjects with Type 2 DM. They found significantly less

healing after NSRCT in subjects with diabetes at the one-year follow up, but not at the 6-month follow up (47).

A critical review of human studies on impact of diabetes on the development of apical periodontitis showed that the quality of evidence presented in the literature was low and no definitive conclusion on the association between diabetes and apical periodontitis can be made (48).

A systematic review of systemic diseases and apical periodontitis revealed only a moderate association between some systemic diseases and endodontic disease. A number of studies that examined the effect of diabetes and cardiovascular disease on development of apical periodontitis contained moderate to high levels of bias (49). Therefore, more prospective longitudinal studies with stronger levels of evidence are needed to investigate the association between the cardiovascular disease, diabetes and endodontic disease (49).

Periapical Index

This prospective study used the periapical index (PAI) introduced by Orstavik in 1986 to rate periapical lesions (50). The PAI is a two-dimensional radiographic scoring system for apical periodontitis with an ordinal scale of five scores from 1(healthy) to 5 (severe apical periodontitis) (51). This system was developed from Brynolf's study that correlated the radiographic interpretation of apical periodontitis to histological findings of extracted human maxillary incisors (52). The thickness of the cortical plate and the position of the root apex to the cortical plate can vary between incisors, canines, pre-molars, and molars (51). Therefore, the validity of using PAI from the results of single rooted teeth in Brynolf's study to score multi-rooted teeth may be questionable (53).

Although Brynolf's histological studies only included incisors, Orastavik argued that the PAI was reproducible and accurate at assessing canines, premolars, and molars (50). The PAI system has been validated in other studies (54, 55). Kirkevang et al. demonstrated that the PAI system was accurate at determining the prognosis of a tooth associated with each of the five scores by following a group of teeth over a 5-year observation period (54). A tooth with a PAI score of 1 had better prognosis than tooth with a PAI score of two which has better prognosis than tooth with a PAI score of three and so on (54). Teeth with higher baseline PAI scores were more likely to be extracted than those with lower PAI scores (54).

Delano et al. (55) found the PAI system correlated well with radiographic assessment of apical periodontitis using an computer model. Objective assessment of apical periodontitis was determined via computer software that calculated the ratio of apical radiolucent area to normal osseous area (AR/N). The AR/N scores were compared between baseline and at multiple follow ups. As healing took place, mean AR/N increased with time, and a significant correlation was found between PAI scores and AR/N indicating the reliability of the PAI for determining apical periodontitis (55).

Purpose

The aim of this study was to evaluate the relationship between HbA1c level and short-term healing of periapical lesions after non-surgical root canal treatment.

Hypothesis

Primary hypothesis:

H_0: There is no relationship between HbA_{1c} level and the healing of periapical lesions after non-surgical root canal treatment.

H_1: There is a relationship between HbA_{1c} level and the healing of periapical lesions after non-surgical root canal treatment.

Secondary hypothesis:

H_0: There is no significant correlation between co-variables and healing of periapical lesions after non-surgical root canal treatment.

H_1: There is a significant correlation between co-variables and healing of periapical lesions after non-surgical root canal treatment.

Tertiary hypothesis:

H_0: There is no significant logistic regression between co-variables and healing of periapical lesions after non-surgical root canal treatment.

H_1: There is a significant logistic regression between co-variables and healing of periapical lesions after non-surgical root canal treatment.

Materials and Methods:

Study Design

This is an ongoing prospective clinical study evaluating the relationship between Hba1c levels and short-term healing of periapical lesions after non-surgical root canal treatment started by previous endodontic resident, Tontesh Tawady (TT), at the University of Maryland, Baltimore (56). Short-term healing is defined as healing less than one-year after root canal treatment. The protocol was approved by the Institutional Review Board (IRB) at the University of Maryland, Baltimore (IRB # HP-00058791) and remained the same for continuation of the study.

Patients referred to the post graduate endodontics clinic and those that meet the inclusion criteria were invited to participate in the study (56). Two endodontic residents (TT) and (ZZ) screened all eligible subjects for participation in the study according to the following inclusion and exclusion criteria (56):

Inclusion Criteria

- Patient must present with tooth diagnosed with pulp necrosis and a periapical lesion measured at least 3mm in diameter.
- Treatment being provided must be initial root canal treatment
- Permanent tooth with closed apices and has a favorable prognosis at the time treatment is rendered.
- Tooth is planned to receive a permanent restoration
- RCT is well-performed:

- Obturation terminates within 0-1mm from the apex in all canals
- No significant voids in obturation
- No missed canals determined from pre-operative and post-operative periapical radiographs

Exclusion Criteria

• Teeth without a treatment plan for a permanent restoration. The referring dentist will place the final restoration unless the tooth requires a simple composite or amalgam that can be placed by the treating endodontic resident.

• Patients diagnosed with a malignancy, liver or kidney disease or any other systemic condition that compromises the immune response.

• Fractured tooth or restoration at recall

Endodontic residents provided standardized root canal treatment for all subjects with teeth diagnosed with pulpal necrosis with symptomatic or asymptotic apical periodontitis and with or without acute or chronic apical abscess.

Study Protocol (56)

Patients referred to the post graduate endodontic clinic for root canal treatments were evaluated for eligibility for the study. Each patient had the following recorded: periodontal probing depth, clinical attachment level, percussion and palpation sensitivity, and pulpal vitality result. Periapical radiographs were also taken, and if a periapical lesion was identified, the patient was given the opportunity to take part in the study. Once the patient agreed to enroll in the study, a research team member (ZZ, PT, PC, TT) who did

not provide the root canal treatment consented the patient into the study. Appropriate IRB consent and HIPPA forms were reviewed and signed.

Periodontal disease status was determined by periodontal probing depth (PD) and clinical attachment level (CAL). Periodontitis is defined according to parameters set forth by Borrell et al. (57) as dentition with a minimum of 3 sites showing CAL ≥ 4mm and at least two sites with PD ≥ 3mm. Both CAL and PD need not be on the same tooth.

A calibration exercise was conducted, using 100 images provided by Dr. Orstavik, represented different stages of periapical disease. Figure 1 shows the scoring system developed by Dr. Orstavik. The PAI scores are based on the correlation between periapical lesions of human cadaver teeth and histology findings which are shown in Table 1 (51, 52). The calibration exercise resulted in a weighted Kappa of .77. Therefore, allowing the study team to score the enrolled subjects' periapical lesion status at baseline and at recall.

Figure 1. Reference Image for PAI scoring (50)

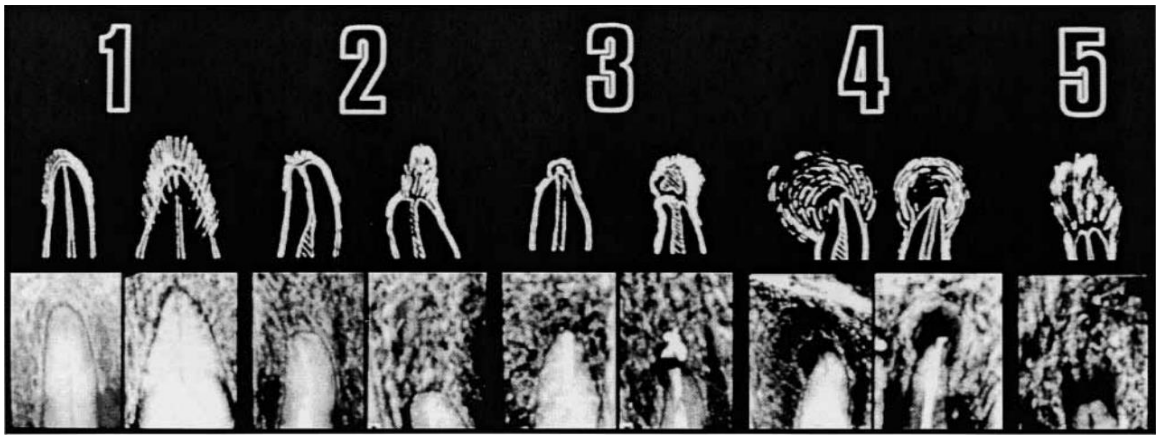


Table 1. PAI score and corresponding description

PAI Score	Histologic Description
1	Normal periapical structures or normal apical periodontium
2	Small changes in periapical bone structure or bone structural changes indicating, but not pathognomonic for, apical periodontitis
3	Changes in periapical bone structure with some mineral loss or bone structural changes with some mineral loss characteristic of apical periodontitis
4	Demineralization of periapical bone with well-defined radiolucent area or well-defined radiolucency
5	Demineralization of periapical bone with exacerbating features or radiolucency with radiating expansions of bone structural changes

After completing clinical and radiographic exams, an endodontic resident initiated non-surgical root canal treatment following a standard protocol at the University of Maryland post-graduate Endodontics clinic. All treatments were performed under dental operating microscopes (DOMs). After local anesthesia and rubber damn isolation, access cavities were prepared, working length determination was made with an electronic apex locator. Canals were instrumented with stainless steel handfiles and Vortex Blue ((Tulsa, Dentsply) rotary files, and irrigated with 2.5% sodium hypochlorite (NaOCl). Sterile paper points were used to dry the canals before obturation with gutta percha and AH Plus sealer via the vertical condensation technique. The treating endodontic resident placed the final resin or amalgam restorations in teeth that did not require full coverage restorations. If a crown was required, a sterile sponge or polytetrafluoroethylene (PTFE) tape was placed in the pulp chamber and sealed with Cavit and Fuji IX temporary restorations. Sterile sponge or PTFE was the material of choice over cotton pellet since the cotton pellet did not provide an adequate seal that could lead to bacterial leakage (58).

An immediate post-operative periapical radiograph was taken with a customized stent made of a bite registration material applied to an XCP. Horizontal and vertical alignment of the x-ray sensor was secured as the patients bit onto the XCP embedded within the bite registration material. This allowed a reproducible alignment of the x-ray reducing errors in magnification at subsequent radiographic examination (59). This custom stent was disinfected and stored until the subjects returned for their recall appointment.

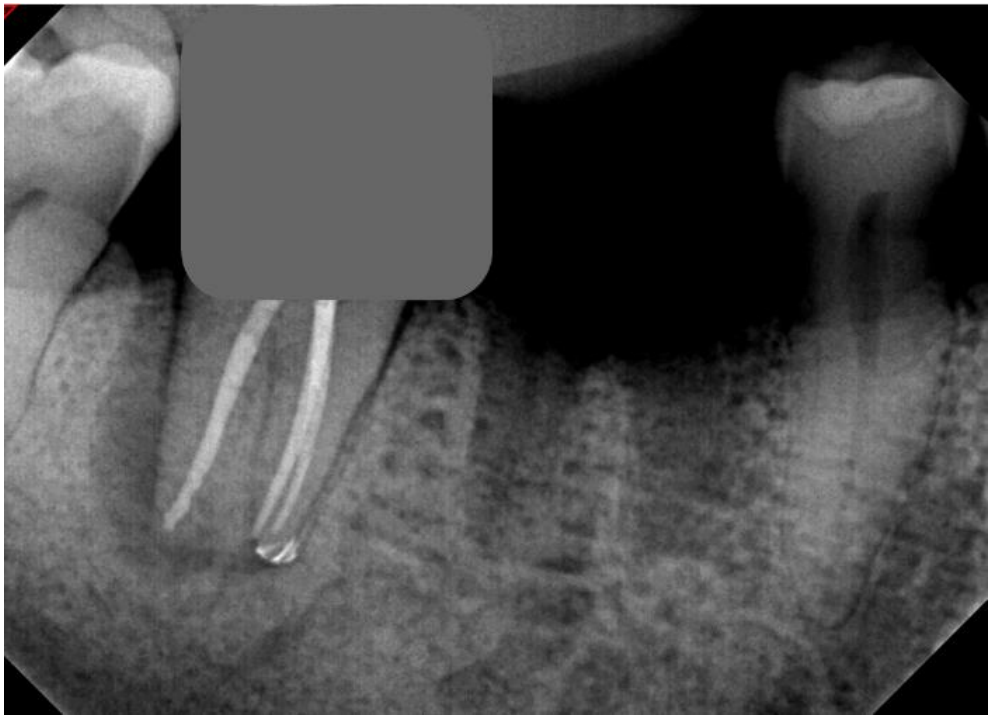
After the immediate post-operative radiograph was taken, a dental school nurse took a blood sample which was sent to University of Maryland Medical System Pathology Laboratory for HbA1c level analysis. Subjects whose HbA1c level returned at 5.7% or higher were referred to their physicians for further examination and evaluation for possible diagnosis of prediabetes or diabetes.

Subjects were recalled for a recall exam six months later. A clinical exam including probing depths, clinical attachment level, percussion and palpation sensitivity was completed. A periapical radiograph using the previously mentioned custom stent was taken. A second blood sample was taken by a dental school nurse and immediately sent to the University of Maryland Medical System Pathology Laboratory for HbA1c analysis. The average of the subjects' baseline and recall HbA1c scores was used in the data analysis.

Every few months, sets of immediate post-operative and recall radiographs were scored. They were presented for scoring in no particular order. This was done so that the rater would not know whether the radiographs being graded were taken at baseline or at recall or which radiographs made a set.

The observer scored the radiographs which were randomized by ZZ through a random number generator and both quality and type of coronal restoration were masked from the observer (Fig 2).

Figure 2. Example of tooth 31 masked on a periapical radiograph



Sample size determination

Sample sizes were determined with Power and Precision™ (60). There was a significant disagreement in the prior research as to whether there was a signification correlation or not between healing of periapical lesions after root canal treatment and the HbA1c level (56). Therefore, it was decided that aa post-hoc analysis would be conducted after data collection was completed. Results of the post-hoc analysis showed that with a sample size of 38 recall subjects, a one-tailed test, and spearman rho of -.52, yielded a power of .90 would be achieved.

Data Analysis

Raw data entered into Microsoft Excel were transferred to the Statistical Package for the Social Sciences (SPSS, Version 25). A paired t-test was used to check for differences between the baseline and recall HbA1c levels in the three groups. Spearman's rho was used to analyze the correlation between HbA_{1c} and the difference between recall and baseline PAI scores (representing healing). Co-variables (age, sex, tooth type, days to recall, cardiovascular disease, hypertension, smoking, and periodontal disease) were compared between groups using Chi-square or Fischer's exact test, as appropriate.

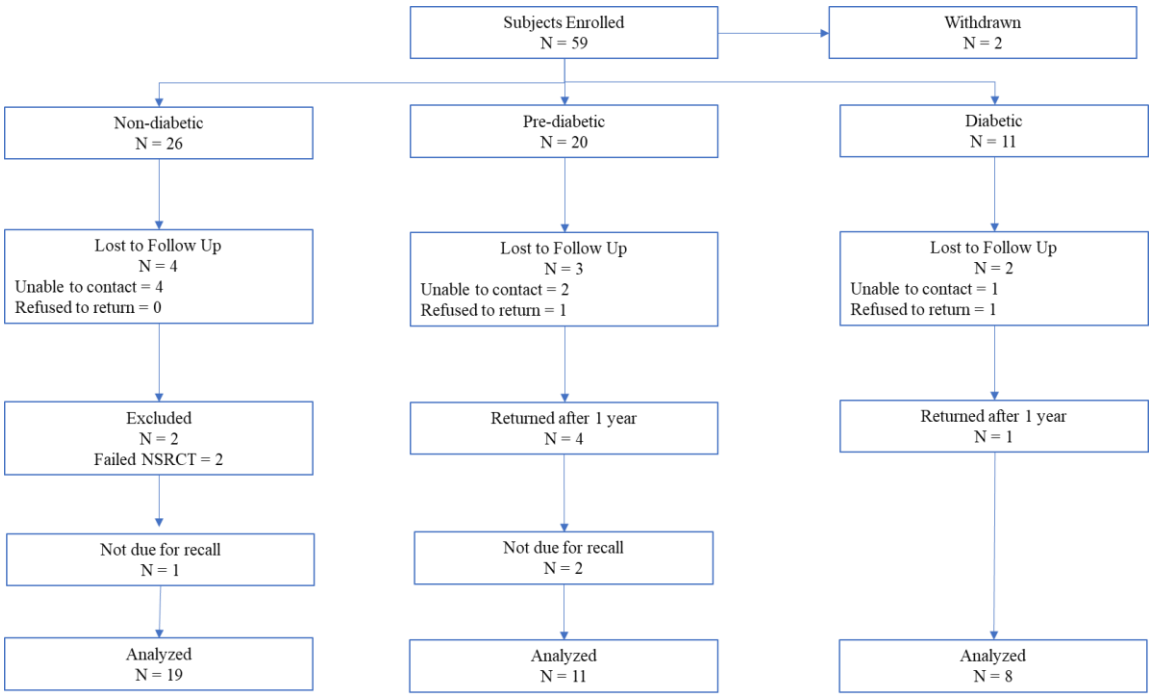
Results

Baseline Data

The flowchart for subject recruitment is shown in Figure 3. Fifty-nine subjects referred to the UMSOD post graduate endodontics clinic were enrolled in this study. Two subjects were withdrawn because they declined to have their blood samples taken for the HbA1c analysis. The results of the baseline demographic characteristics of the 57 subjects are reported in Table 2.

Because of the inability to contact nine subjects, they were lost to follow up. In addition, two subjects had apicoectomies with retrograde fillings due to failing initial root canal treatments before they returned for their recall exams. Both apicoectomies were completed on mesial buccal and distal buccal roots of their maxillary first molars. Five subjects returned for the recall exam after 15 months. The five subjects were not included in this short-term analysis. Three subjects were not due for their recalls at the time of preparing this manuscript.

Figure 3: Flow chart for subject recruitment



Baseline Demographic Characteristics

For baseline comparison between the three groups, Kruskal-Wallis was used to assess age and (Baseline PAI) among normal, prediabetic, and diabetic groups because of the very uneven number of subjects in the three groups. Chi-square or Fisher's Exact test was used to assess the categorical variables (Gender, Tooth type, Periodontal disease, Cardiovascular disease, Hypertension, Smoking status) between the three groups, as appropriate. There were no significant differences between any variables between the three groups at baseline (Table 2).

Table 2. Baseline Demographic Characteristics (N = 57)

	Normal n = 26	Prediabetes n = 20	Diabetes n = 11	Statistical Result	*p*
Age years (mean, SD)	50.62 ± 15.8[a]	64.2 ± 14.6[b]	61.7 ± 11.1[b]	Kruskal-Wallis = 9.641	*p = .008*
Gender:				Fisher's Exact test = 1.638	*p = .43*
Females (n=24)	13 (50%)	8 (67%)	3 (27%)		
Males (n=33)	13 (50%)	12 (23%)	8 (63%)		
Tooth Type:				Fisher's Exact test = 4.764	*p = .32*
Anteriors (n=15)	5 (19%)	6 (30%)	4 (36%)		
Premolars (n=18)	12 (46%)	4 (20%)	2 (18%)		
Molars (n=24)	9 (35%)	10 (50%)	5 (46%)		
Periodontal disease:				Fisher's Exact test = 2.447	*p = .33*
No (n=38)	20 (77%)	12 (60%)	6 (55%)		
Yes (n=19)	6 (23%)	8 (40%)	5 (45%)		
Hypertension:				Fisher's Exact test = 5.817	*p = .05*
No (n=35)	20 (77%)	11 (55%)	4 (36%)		
Yes (n=22)	6 (23%)	9 (45%)	7 (64%)		
Cardiovascular disease:				Fisher's Exact test = 2.646	*p = .32*
No (n=51)	25 (96%)	17 (85%)	9 (82%)		
Yes (n=6)	1 (4%)	3 (15%)	2 (18%)		
Smoking status:				Fisher's Exact test = .298	*p = 1.0*
No (n=49)	22 (85%)	17 (85%)	10 (91%)		
Yes (n=8)	4 (15%)	3 (15%)	1 (9%)		
Baseline PAI (mean)	4.0	3.5	3.3	Kruskal-Wallis = 5.004	*p = .082*

* Different letters indicate significant differences

Recall Results

Of the 59 subjects enrolled in this study, 38 returned for short-term recall. There were no significant differences between baseline and recall HbA1c levels among all three groups (Table 3). Of the 38 recalled subjects, a significant decrease was found between baseline and recall PAI scores (3.61 and 2.26, matched pairs t-test = 6.264, $p \leq .0005$, Table 4).

Table 3: Baseline and Recall HbA1c level of recall subjects

	Baseline HbA1c mean	**Recall HbA1c mean**	Statistical Result	*p*
Normal HbA1c n = 19	5.41	5.35	Matched pairs t-test = .959	*p=.35*
Prediabetes n = 11	5.94	5.81	Matched pairs t-test = 1.617	*p=.13*
Diabetes n = 8	7.55	7.84	Matched pairs t-test = -1.51	*p=.17*

Table 4: Recall subjects: Baseline and Recall PAI

Baseline PAI (mean)	Recall PAI (mean)	Statistical Result	*p*
3.61	2.26	Matched pairs t-test = 6.264	$p \leq .0005$

For recall subject comparison, a Kruskal-Wallis test was used to assess (age, recall days, recall PAI, and PAI difference) between normal, pre-diabetes, and diabetes groups. Chi-square or Fisher's exact test was used to assess gender, tooth type, periodontal disease, hypertension, cardiovascular disease, and smoking status among the three groups. There were no significant differences between all tested variables except for age and PAI difference among the three groups (Table 5).

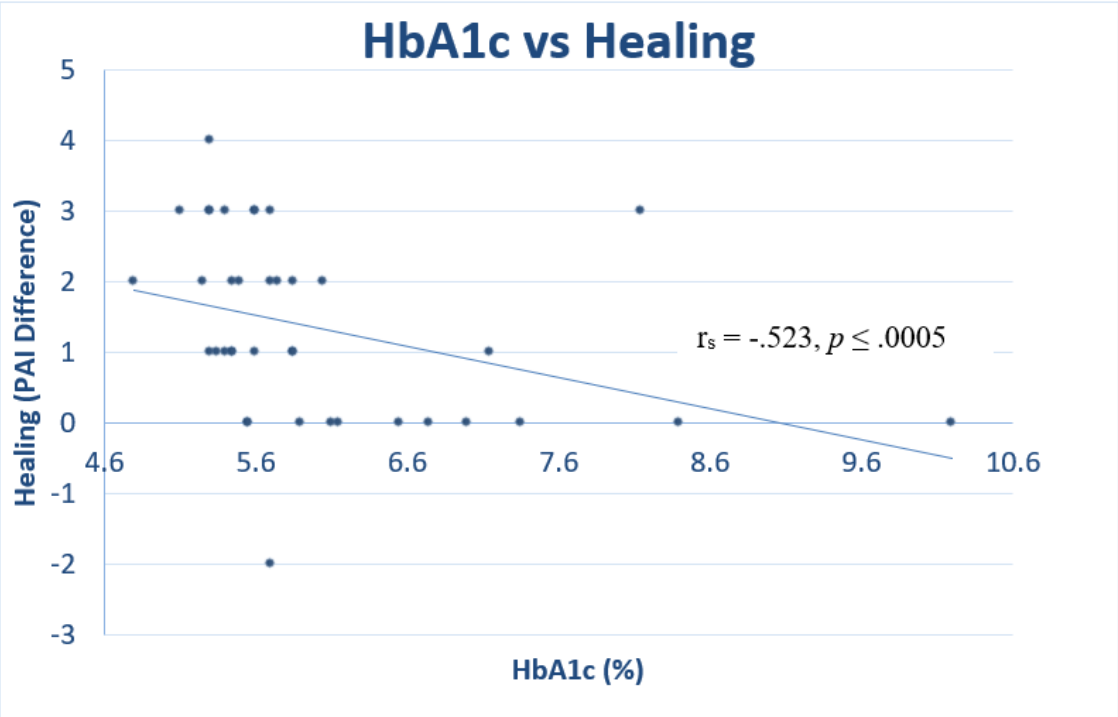
Table 5: Recall subjects' comparison

	Normal n = 19	Prediabetes n = 11	Diabetes n = 8	Statistical Result	*p*
Age years (mean, ± SD)	49.3 ± 15.6[a]	63.6 ± 10.9[b]	64 ± 7.7[b]	Kruskal-Wallis = 8.284	*p = .02*
Gender:				Fisher's exact test = 3.716	*p = .18*
Females (n=16)	10 (53%)	5 (45%)	1 (12%)		
Males (n=22)	9 (47%)	6 (55%)	7 (87%)		
Recall days (mean)	203	207	213	Kruskal-Wallis = .632	*p = .729*
Tooth Type:				Fisher's exact test = 4.488	*p = .361*
Anterior (n=10)	4 (21%)	3 (27%)	3 (37%)		
Premolar (n=12)	9 (47%)	2 (18%)	1 (12%)		
Molar (n=16)	6 (32%)	6 (55%)	4 (51%)		
Periodontal disease:				Fisher's exact test = 1.077	*p = .554*
No (n=24)	12 (63%)	8 (73%)	4 (50%)		
Yes (n=14)	7 (37%)	3 (27%)	4 (50%)		
Hypertension:				Fisher's exact test = 2.423	*p = .245*
No (n=26)	15 (79%)	7 (64%)	4 (50%)		
Yes (n=12)	4 (21%)	4 (26%)	4 (50%)		
Cardiovascular disease:				Fisher's exact test = 2.693	*p = .243*
No (n=36)	19 (100%)	10 (91%)	7 (88%)		
Yes (n=2)	0 (0%)	1 (9%)	1 (12%)		
Smoker:				Fisher's exact test = 2.447	*p = .384*
No (n=33)	15 (79%)	11 (100%)	7 (88%)		
Yes (n=5)	4 (21%)	0 (0%)	1 (12%)		
Baseline PAI (mean)	3.88	3.36	3.25	Kruskal-Wallis = 2.929	*p = .231*
Recall PAI (mean)	1.94	2.36	2.75	Kruskal-Wallis = 1.50	*p = .472*
PAI difference (mean)	1.94	1	0.5	Kruskal-Wallis = 8.11	*p = .02*

* Different letters indicate significant differences

Spearman's rho revealed a significant negative correlation between HbA1c and healing (r_s = -.523, $p \leq .0005$) (Figure 4, Table 6).

Figure 4: Correlation between healing (PAI Difference) and HbA1c levels



Correlation between co-variables and healing

To assess possible relationships between the co-variables (Gender, Tooth type, Periodontal disease, Cardiovascular disease, Hypertension, Smoking status, HbA1c, age, and recall days) and healing (PAI difference), Spearman's rho was completed. Significant correlations were found between healing and HbA1c (r_s = -.523, $p \leq .0005$), and healing and cardiovascular disease (r_s = -.34, $p \leq .01$), healing and age (r_s = -.44, $p \leq .003$), healing and recall days (r_s = .29, $p \leq .04$), age and HbA1c (r_s = .46, $p \leq .002$), age and cardiovascular disease (r_s = .36, $p \leq .013$), and cardiovascular disease and recall days (r_s = -.36, $p \leq .031$) (Table 6).

Table 6: Spearman's rho correlation between co-variables and healing

		HbA1c	CVD	Age	Recall days
Spearman's rho	PAI Difference	-.52* ($p \leq .0005$)	-.34* ($p \leq .01$)	-.44* ($p \leq .003$)	.29* ($p \leq .04$)
	CVD	.22			-.36* ($p \leq .031$)
	Age	.46* ($p \leq .002$)	.36* ($p \leq .013$)		-.12

* Indicates statistically significant values

Logistic regression

While a significant correlation between age and the HbA1c level was found, subjects with higher HbA1c levels were significantly older than those with normal HbA1c, and thus age was dropped out of the multivariate logistic regression analysis. Since cardiovascular disease was significantly correlated with age, it was also dropped out of the logistic regression analysis. Therefore, a final logistic regression was run with HbA1c level, recall days and PAI difference. This test revealed a significant relationship between HbA1c level and PAI difference (Table 7). The total logistic regression R value of .62 indicates that the HbA1c level and recall days contributed to 62% of the healing of periapical lesions after non-surgical root canal treatment.

Table 7: Multivariate logistic regression analysis of independent variables (HbA1c and recall days) on PAI difference.

Dependent variable (Healing)	Total logistic regression R	Independent variables	B value	*p*
PAI Difference (1)	.62	HbA1c	3.366	*p=.015*
		Recall (days)	-0.019	*p=.315*

* Indicates a statistically significant correlation between healing and HbA1c and recall days.

Discussion

The aim of this prospective clinical study was to examine the relationship between HbA1c levels and healing of periapical lesions after non-surgical root canal treatment. Baseline demographic characteristics related to gender, age, days to recall, smoking status, cardiovascular disease, periodontal disease, and hypertension status of the participants were statistically similar in all groups.

Diabetic status was established according to current guidelines by the American Diabetic Association (16). The glycated Hemoglobin (HbA1c) test provides an objective blood glucose level over the previous eight to twelve weeks and is recommended by an International Expert Committee and the American Diabetes Association as a means to diagnose diabetes (18, 61). Patients with diabetes present with elevated HbA1c formed through a covalent enzymatic reaction between glucose and a minor variant of hemoglobin that is associated with elevated blood glucose (15, 19).

According to the Center for Disease Control (CDC), a diabetes diagnosis increases with age (2). In this study, the mean age of subjects who completed the study and had

HbA1c levels in the diabetic range were 64 years old compared to the mean age of those whose HbA1c levels were normal (44 years old).

This study used the PAI scoring system developed by Orstavik to assess healing after root canal treatment (50). The PAI system relies on evidence from an earlier study by Brynolf who found a high correlation between the degree of apical periodontitis on periapical radiographs and histological changes (50, 52). Although Brynolf exclusively studied incisors, Orstavik found strong intra- and inter-observer agreement in multirooted teeth, indicating that the PAI might be accurate for the entire dentition. This result has been validated by other studies (50, 54, 55).

To minimize the effect of the co-variables, we applied inclusion and exclusion criteria. Support for these decisions, when not obvious are described as follows. Patients diagnosed with malignancies (except those with diabetes) were excluded from the present study. Also, root canal obturation was required to be of high quality terminating, within 2mm from the radiographic apex and without voids (62). At recall, all teeth had to have permanent restorations. The quality of the coronal seal could impact the outcome of root canal treatment (63). Teeth with adequate root canal treatments and without adequate coronal restorations have been shown to have significantly lower healing rates (63, 64). Temporary restorations, such as Fuji, leak after three months, thereby recontaminating the root canal treatment (65, 66).

In our study, there were significant differences in age between the three groups in the baseline demographic characteristics. These differences in age might have occurred because of the uneven sample sizes between the three groups. There were 26 normal

subjects, but only 20 subjects with prediabetes, and 11 subjects with diabetes. There was also a significant difference in those diagnosed with hypertension between the groups. There were more subjects diagnosed with hypertension in the diabetes group compared to the other two groups. Perhaps this was because diabetes is both a metabolic disorder of glucose metabolism as well as a vascular disease. Hypertension is common amongst diabetics (3, 67). All other baseline demographic characteristic variables were not significantly different between the three groups.

In our study, there was a significant negative correlation (r_s = -.523) between the HbA1c level and healing of the periapical lesion after root canal treatment. The level of healing is determined quantitatively by subtracting the recall PAI from the baseline PAI score. A small difference in the PAI scores represents less healing while larger differences represent greater healing. The results showed subjects with higher HbA1c levels had smaller short-term healing than those with lower or normal HbA1c levels. This suggests, that glucose metabolism influenced the healing rate of these periapical lesions.

Although the r_s is typically presented in a research paper, it is the r_s^2 that is interpreted (r_s^2 = .27). Our results, imply that about 27% of the healing is attributed to the HbA1c level leaving 73% of the healing to other variables such as the subjects' age or tooth anatomy. Teeth in older subjects have more calcified tissue which is more difficult to thoroughly debride. This definitely could impact healing (68). The anatomy of the teeth might affect the healing after root canal treatment because multiple rooted teeth, such as molars, have isthmuses that harbor bacteria (69). Isthmuses are likely not accessible to the passive irrigation and rotary filing in a conical shape which is the standard cleaning and

shaping technique in modern endodontics. This increases the potential of leaving bacteria and bacterial by-products in the canal and can reduce the level of healing (69).

Significant correlations were also found between age, cardiovascular disease, and recall time with healing. While few studies found no significant impact of age on outcome of NSRCT (62, 70), Lee et al. (71) found patients younger than 50 years old showed more favorable periapical healing after non-surgical root canal treatments. This might be due to an increase of pulp stones, secondary/tertiary dentin deposition, and dystrophic calcifications in the older population. Pulp stones pose a challenge to adequate debridement of the root canal system leading to reduced healing (71).

Age was also significantly correlated with HbA1c. This is expected because diabetes is usually found in older populations (2). In this study, subjects with higher HbA1c levels were significantly older than those with normal HbA1c values. Therefore, age was dropped from the logistic regression analysis. Since CVD was also correlated with age, CVD was dropped from the logistic regression analysis. A significant correlation was also found between cardiovascular disease and days to recall. This was most likely due to the small sample size since we should expect no relationship between cardiovascular disease and recall time.

A considerable effort was made to recall all subjects exactly six months after root canal treatment. However, some were unable to return on the sixth month. Reasons included traveling outside of the country, wanting to combine the recall appoint with another dental appointment, or other obligations that necessitated them to return after the recommended six months periods. The recall times ranged between six and nine months.

Subjects with longer recall times showed better healing, which might have affected the correlation between HbA1c level and healing. However, a final logistic regression analysis of HbA1c level and recall time on healing showed only the HbA1c level was significantly related healing.

The HbA1c levels were measured immediately after completion of NSRCT at both baseline and at recall periods. In all three groups, there were no significant changes in HbA1c level between the two time points indicating stable glycemic control. However, subjects in the prediabetes group showed a small decrease in their recall HbA1c levels. This may be attributed to a recommendation that they follow-up with their primary care physician for necessary medical management. Another reason for the improvement in glycemic control might have been the result of the root canal treatment itself. Schulze et al.(72) reported a case of sudden improvement in insulin sensitivity in a patient after root canal treatment of an abscessed tooth. The removal of necrotic pulp tissue decreases the level of endotoxin that could systemically circulate, reducing inflammation and leading to improved glycemic control (72).

Diabetes weakens the immune system by reducing functional macrophages involved with chemotaxis, phagocytosis and microorganism killing. It depresses polymorphonuclear neutrophil (PMN) function leading to increased infection susceptibility (8, 73, 74). Diabetes promotes the release of pro-inflammatory cytokines from PMNs and down-regulates growth factor release from macrophages. As a result, diabetics are predisposed to chronic infection, inflammation and reduced tissue repair (75). This may explain why the subjects with higher HbA1c levels had slower periapical tissue healing compared to those with normal HbA1c levels. In addition to periapical delayed

healing, chronic inflammation delays pulpal tissue healing (13). Proinflammatory cytokines IL-1b , IL-6, and tumor necrosis factor a (TNF-a) promote osteoclastic activity leading to bone resorption through modification of the receptor activator of the nuclear factor kappa-B (RANK) or the RANK ligand (76). Chronic inflammation can elevate blood glucose levels that require insulin and therapeutic adjustments (12). Therefore reduction in overall inflammation may improve glycemic control (12).

Elevated blood glucose is not only associated with a heightened level of inflammation but also interacts with proteins, amino acids, and lipids to form irregular shaped advanced glycation end products (AGEs) that contribute to comorbidities associated with diabetes (10). Proinflammatory cytokines (IL-1b, IL-6, and IL-8, TNF-a, and PG E2), when circulating though the vascular system, might interact with AGEs (47). Activation of inflammation in immune, muscle, fat, endothelium cells, and adipocytes contribute to insulin resistance that alters metabolic control in patients with Type 2 DM and apical periodontitis (77, 78). This may negatively influence periapical lesion healing in patients with poor glycemic control. Furthermore, association of AGE with its receptors, leads to an activated cellular response inhibiting bone mineralization while promoting bone resorption (79). Even after root canal treatment, boney healing of periapical lesions can be affected.

Various clinical studies have analyzed the relationship between diabetes, periapical status, NSRCT outcomes (48). Some studies state patients with diabetes display poorer healing after NSRCT in teeth with periapical lesions. Cheraskin and Ringsdorf found subjects with lower blood glucose levels showed more reduction in the size of a periapical lesion after NSRCT, compared to subjects with higher blood glucose levels (41). Sánchez-

Domínguez and others found HbA1c levels ≥ 6.5% were correlated with an increased prevalance of apical periodontitis (45). Results of the present study showed subjects with higher HbA1c levels at 6-8 month follow-up had smaller declines in PAI scores than those with lower HbA1c levels. Our findings support both of these earlier investigations.

Fouad and Burleson's retrospective study found diabetes reduced the success of NSRCT in teeth presenting with pre-operative periapical lesions (44). A longitudinal study by Arya and others found that patients with diabetes had significantly lower NSRCT success at the one-year follow-up, despite finding difference at the six-month follow-up (47). These studies support the present study which found that subjects with higher HbA1c levels had less healing than those with lower HbA1c levels.

Falk and others (42) and Britto and others (43) reported subjects with diabetes were more likely to have residual lesions after root canal treatment. Unlike our study that considered pre-operative periapical lesion as an inclusion criterion, Falk and Britto did not disclose whether periapical lesions were present pre-operatively. Therefore, we do not know if the periapical lesions developed after the completion of NSRCT. Furthermore, our study used digital periapical radiographs to assess periapical tissues, while the others used panoramic radiographs, which underestimated the size of periapical lesions (80). Periapical radiography is more accurate at detecting periapical lesions compared to panoramic radiography, particularly in in the maxillary premolar and mandibular molar regions (80).

Marotta and others found no difference in the prevalence of periapical lesions associated with root canal treated teeth in patients with and without diabetes (46). Their findings did not agree with our results in which subjects with higher HbA1c levels had

significantly less healing than those with lower or normal HbA1c levels. The differences could be attributed to the study design. This study was longitudinal, while the other was cross-sectional and periapical status before and immediately after root canal treatment was not recorded, so the PAI difference could not be determined (48).

Subjects in this study were recalled between six and eight months after completion of NSRCT. Reit and others recommended one-year follow-up after NSRCT (81). Wu and Wesselink recommended waiting two years after completion of initial treatment before declaring presence or absence of post-treatment disease. Our recall time was chosen to maximize patient compliance and to comply with the time restrictions of an endodontic residency program.

Thirty-seven subjects who returned for recall showed improved PAI scores or no change. This revealed that periapical healing could be seen within one year. Only one subject had a higher PAI score at follow-up. The subject had treatment on a mandibular, right second molar which developed a furcation radiolucency. Furcation canals are found about 29.4% of the time in mandibular molars and harbors necrotic tissue and bacteria (82). If these were left un-instrumented, bacteria in these furcation canals could cause bone loss leading to development of bony lesions. Another possibility for non-healing would be a vertical root fracture. The other 37 recall subjects showed no change or improved PAI scores.

Limitations

Limitations of this study included a small diabetic group sample size and low recall rate. Of the 59 subjects enrolled, only 38 completed the study: eight subjects with diabetes,

11 with prediabetes, and 19 non-diabetic controls. All participants in this study were compensated for being enrolled in the study. They were further compensated for returning for their recall visit. However, only 64% of the participants returned for their short-term recall. Some did not come back due to being asymptomatic or for living too far from the dental school to make a second trip worthwhile. Others moved away or had disconnected phone numbers. However, other outcome studies incorporating monetary incentives for recalls achieved even lower rates of 43% and 50% (83, 84).

Another limitation of this study was the use of two-dimensional radiography to assess periapical lesions. Cone-beam tomography (CBT) allows precise evaluation of the true extent of the periapical lesion compared to conventional periapical radiography (85). Significantly more periapical lesions are missed in periapical radiography as compared to CBT, especially in the maxillary posterior regions due to the close proximity of roots to the maxillary sinus (85).

The short-term recall of six to eight months after root canal treatments is a third limitation in this study. In general, patients are asked by their endodontist to return for their follow up exams after one or two years (81, 86). This is because post-treatment disease can persist, requiring further surgical or non-surgical intervention, despite the reduction in the size of the periapical lesions.

Future Direction

Before CBT came into dentistry, PAI, using periapical radiography, was the primary method of analyzing healing in outcome studies (54, 55). Recent studies have found a positive correlation between the volume of the apical lesions assessed by CBT and

the PAI (87). Estrela et al. developed a revised PAI based on CBCT (CBCTPAI) that provides a more accurate diagnosis. CBCTPAI uses higher resolution images and decreases the incidence of false-negatives, which increased overall reliability compared to two-dimensional periapical radiography (88). Therefore, future studies should incorporate CBT based PAI to improve diagnostic accuracy for detecting apical periodontitis.

Conclusion

There was a significant negative correlation between HbA1c levels and short-term healing of periapical lesions after non-surgical root canal treatment. There was also a significant correlation between healing and cardiovascular disease, healing and age, healing and days to recall, age and HbA1c, and age and cardiovascular disease. A final logistic regression showed a significant relationship between HbA1c level and short-term healing of periapical lesions.

References

1. Wilcox G. Insulin and insulin resistance. The Clinical Biochemist. Reviews 2005;26(2):19-39.
2. Newswire PR. New CDC report: More than 100 million Americans have diabetes or prediabetes. In: CDC-diabetes-report. Y; 2017.
3. Diagnosis and classification of diabetes mellitus. Diabetes Care 2014;37 Suppl 1:S81-S90.
4. Rowley WR, Bezold C, Arikan Y, Byrne E, Krohe S. Diabetes 2030: Insights from Yesterday, Today, and Future Trends. Population health management 2017;20(1):6-12.
5. Ahmadieh H, Azar ST. The role of incretin-based therapies in prediabetes: A review. Primary Care Diabetes 2014;8(4):286-294.
6. Nathan DM, Genuth S, Lachin J, Cleary P, Crofford O, Davis M, et al. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. New England Journal of Medicine 1993;329(14):977-986.
7. Bryan CS, Reynolds KL, Metzger WT. Bacteremia in Diabetic Patients: Comparison of Incidence and Mortality with Nondiabetic Patients. Diabetes Care 1985;8(3):244.
8. Joshi N, Caputo GM, Weitekamp MR, Karchmer AW. Primary care: infections in patients with diabetes mellitus. New England Journal of Medicine 1999;341(25):1906-1912.
9. Muchová J, Liptáková A, Országhová Z, Garaiová I, Tison P, Cársky J, et al. Antioxidant systems in polymorphonuclear leucocytes of Type 2 diabetes mellitus. Diabetic Medicine: A Journal Of The British Diabetic Association 1999;16(1):74-78.
10. Ahmed N. Review: Advanced glycation endproducts—role in pathology of diabetic complications. Diabetes Research and Clinical Practice 2005;67:3-21.
11. Lima SMF, Grisi DC, Kogawa EM, Franco OL, Peixoto VC, Gonçalves-Júnior JF, et al. Diabetes mellitus and inflammatory pulpal and periapical disease: a review. International Endodontic Journal 2013;46(8):700-709.
12. Bender IB, Bender AB. Diabetes Mellitus and the Dental Pulp. Journal of Endodontics 2003;29(6):383-389.
13. Garber SE, Shabahang S, Escher AP, Torabinejad M. The Effect of Hyperglycemia on Pulpal Healing in Rats. Journal of Endodontics 2009;35(1):60-62.
14. Dienelt A, zur Nieden NI. Hyperglycemia impairs skeletogenesis from embryonic stem cells by affecting osteoblast and osteoclast differentiation. Stem Cells And Development 2011;20(3):465-474.
15. Peterson KP, Pavlovich JG, Goldstein D, Little R, England J, Peterson CM. What is hemoglobin A1c? An analysis of glycated hemoglobins by electrospray ionization mass spectrometry. Clinical Chemistry 1998;44(9):1951.
16. Chamberlain JJ, Rhinehart AS, Shaefer Jr CF, Neuman A, Shaefer CF, Jr. Diagnosis and Management of Diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. Annals of Internal Medicine 2016;164(8):542.
17. Bonora E, Tuomilehto J. The Pros and Cons of Diagnosing Diabetes With A1C. Diabetes Care 2011;34:S184-S190.
18. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia 2007;50(11):2239-2244.
19. Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin AIc in diabetes mellitus. The New England journal of medicine 1976;295(8):417-420.

20. Saudek CD, Herman WH, Sacks DB, Bergenstal RM, Edelman D, Davidson MB. A new look at screening and diagnosing diabetes mellitus. The Journal Of Clinical Endocrinology And Metabolism 2008;93(7):2447-2453.
21. Leow MKS. Glycated Hemoglobin (HbA1c): Clinical Applications of a Mathematical Concept. Acta Informatica Medica: AIM: Journal Of The Society For Medical Informatics Of Bosnia & Herzegovina: Casopis Drustva Za Medicinsku Informatiku Bih 2016;24(4):233-238.
22. Sznajder N, Carraro JJ, Rugna S, Sereday M. Periodontal findings in diabetic and nondiabetic patients. Journal Of Periodontology 1978;49(9):445-448.
23. Hugoson A, Thorslensson H, Falk H, Kuylenstierna J. Periodontal conditions in insulin-dependent diabetics. Journal of Clinical Periodontology 1989;16(4):215.
24. Emrich LJ, Shlossman M, Genco RJ. Periodontal disease in non-insulin-dependent diabetes mellitus. Journal Of Periodontology 1991;62(2):123-131.
25. Kaur G, Holtfreter B, Rathmann WG, Schwahn C, Wallaschofski H, Schipf S, et al. Association between type 1 and type 2 diabetes with periodontal disease and tooth loss. Journal of Clinical Periodontology 2009;36(9):765-774.
26. Grossi SG, Skrepcinski FB, DeCaro T, Robertson DC, Ho AW, Dunford RG, et al. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. Journal Of Periodontology 1997;68(8):713-719.
27. Sun W-L, Chen L-L, Zhang S-Z, Wu Y-M, Ren Y-Z, Qin G-M. Inflammatory cytokines, adiponectin, insulin resistance and metabolic control after periodontal intervention in patients with type 2 diabetes and chronic periodontitis. Internal Medicine (Tokyo, Japan) 2011;50(15):1569-1574.
28. Janket SJ, Wightman A, Baird AE, Van Dyke TE, Jones JA. Does periodontal treatment improve glycemic control in diabetic patients? A meta-analysis of intervention studies. Journal Of Dental Research 2005;84(12):1154-1159.
29. Engebretson SP, Hyman LG, Michalowicz BS, Schoenfeld ER, Gelato MC, Hou W, et al. The effect of nonsurgical periodontal therapy on hemoglobin A1c levels in persons with type 2 diabetes and chronic periodontitis: a randomized clinical trial. JAMA 2013;310(23):2523-2532.
30. Caplan DJ, Chasen JB, Krall EA, Cai J, Kang S, Garcia RI, et al. Lesions of endodontic origin and risk of coronary heart disease. Journal of dental research 2006;85(11):996-1000.
31. Liljestrand JM, Mäntylä P, Paju S, Buhlin K, Kopra KAE, Persson GR, et al. Association of Endodontic Lesions with Coronary Artery Disease. Journal of Dental Research 2016;95(12):1358-1365.
32. Wang C-H, Chueh L-H, Chen S-C, Feng Y-C, Hsiao CK, Chiang C-P. Impact of diabetes mellitus, hypertension, and coronary artery disease on tooth extraction after nonsurgical endodontic treatment. Journal Of Endodontics 2011;37(1):1-5.
33. Costa CPS, Thomaz EBAF, Souza SdFC. Clinical Research: Association between Sickle Cell Anemia and Pulp Necrosis. Journal of Endodontics 2013;39:177-181.
34. Sabeti M, Slots J. Herpesviral-bacterial coinfection in periapical pathosis. Journal Of Endodontics 2004;30(2):69-72.
35. Duncan HF, Pitt Ford TR. The potential association between smoking and endodontic disease. International Endodontic Journal 2006;39(11):843-854.
36. Segura-Egea JJ, Jiménez-Pinzón A, Ríos-Santos JV, Velasco-Ortega E, Cisneros-Cabello R, Poyato-Ferrera MM. High prevalence of apical periodontitis amongst smokers in a sample of Spanish adults. International Endodontic Journal 2008;41(4):310-316.
37. Iwama A, Nishigaki N, Nakamura K, Imaizumi I, Shibata N, Yamasaki M, et al. The Effect of High Sugar Intake on the Development of Periradicular Lesions in Rats with Type 2 Diabetes. Journal of Dental Research 2003;82(4):322.

38. Garber SE, Shabahang S, Escher AP, Torabinejad M. Basic research—biology: The Effect of Hyperglycemia on Pulpal Healing in Rats. Journal of Endodontics 2009;35:60-62.
39. Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-capping material. Journal Of The American Dental Association (1939) 1996;127(10):1491-1494.
40. Moutschen MP, Scheen AJ, Lefebvre PJ. Impaired immune responses in diabetes mellitus: analysis of the factors and mechanisms involved. Relevance to the increased susceptibility of diabetic patients to specific infections. Diabete & Metabolisme 1992;18(3):187-201.
41. Cheraskin E, Ringsdorf WM, Jr. The biology of the endodontic patient. 3. Variability in periapical healing and blood glucose. Journal Of Oral Medicine 1968;23(3):87-90.
42. Falk H, Hugoson A, Thorstensson H. Number of teeth, prevalence of caries and periapical lesions in insulin-dependent diabetics. European Journal of Oral Sciences 1989;97(3):198.
43. Britto LR, Katz J, Guelmann M, Heft M. Periradicular radiographic assessment in diabetic and control individuals. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontology 2003;96(4):449-452.
44. Fouad AF, Burleson J. The effect of diabetes mellitus on endodontic treatment outcome: data from an electronic patient record. Journal of the American Dental Association (JADA) 2003;134(1):43-120.
45. Sánchez-Domínguez B, López-López J, Jané-Salas E, Castellanos-Cosano L, Velasco-Ortega E, Segura-Egea JJ. Glycated hemoglobin levels and prevalence of apical periodontitis in type 2 diabetic patients. Journal Of Endodontics 2015;41(5):601-606.
46. Marotta PS, Fontes TV, Armada L, Lima KC, Rôças IN, Siqueira JF, Jr. Type 2 diabetes mellitus and the prevalence of apical periodontitis and endodontic treatment in an adult Brazilian population. Journal Of Endodontics 2012;38(3):297-300.
47. Arya S, Duhan J, Tewari S, Sangwan P, Ghalaut V, Aggarwal S. Healing of Apical Periodontitis after Nonsurgical Treatment in Patients with Type 2 Diabetes. Journal Of Endodontics 2017;43(10):1623-1627.
48. Tibúrcio-Machado CdS, Bello MDC, Maier J, Wolle CFB, Bier CAS. Review Article: Influence of Diabetes in the Development of Apical Periodontitis: A Critical Literature Review of Human Studies. Journal of Endodontics 2017;43:370-376.
49. Khalighinejad N, Aminoshariae MR, Aminoshariae A, Kulild JC, Mickel A, Fouad AF. Association between Systemic Diseases and Apical Periodontitis. Journal of Endodontics 2016;42(10):1427-1434.
50. Ørstavik D, Kerekes K, Eriksen HM. The periapical index: A scoring system for radiographic assessment of apical periodontitis. Dental Traumatology 1986;2(1):20.
51. Huumonen S, Ørstavik D. Radiological aspects of apical periodontitis. Endodontic Topics 2002;1(1):3.
52. Brynolf I. A histological and Roentgenological study of the periapical region of human upper incisors. 1967.
53. Wu MK, Shemesh H, Wesselink PR. Limitations of previously published systematic reviews evaluating the outcome of endodontic treatment. International Endodontic Journal 2009;42(8):656-666.
54. Kirkevang LL, Ørstavik D, Wenzel A, Vaeth M. Prognostic value of the full-scale Periapical Index. International Endodontic Journal 2015;48(11):1051-1058.
55. Delano EO, Ludlow JB, Ørstavik D, Tyndall D, Trope M. Oral and Maxillofacial Radiology: Comparison between PAI and quantitative digital radiographic assessment of apical healing after

endodontic treatment. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology 2001;92:108-115.
56. Tawady TS. The Relationship between Short Term Healing of Periapical Lesions and Glycemic Control. 2016.
57. Borrell LN, Burt BA, Taylor GW. Prevalence and trends in periodontitis in the USA: the [corrected] NHANES, 1988 to 2000. Journal Of Dental Research 2005;84(10):924-930.
58. Paranjpe A, Jain S, Alibhai KZ, Wadhwani CP, Darveau RP, Johnson JD. In vitro microbiologic evaluation of PTFE and cotton as spacer materials. Quintessence International 2012;43(8):703-707.
59. Pettiette MT, Delano EO, Trope M. Evaluation of success rate of endodontic treatment performed by students with stainless-steel K-files and nickel-titanium hand files. Journal Of Endodontics 2001;27(2):124-127.
60. Borenstein M RH, Cohen J. Power and Precision. Englewood, NJ: Biostat Inc; 2001.
61. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009;32(7):1327-1334.
62. Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K. Outcome of primary root canal treatment: systematic review of the literature – Part 2. Influence of clinical factors. International Endodontic Journal 2008;41(1):6-31.
63. Ray HA, Trope M. Periapical status of endodontically treated teeth in relation to the technical quality of the root filling and the coronal restoration. International Endodontic Journal 1995;28(1):12-18.
64. Tronstad L, Asbjornsen K, Doving L, Pedersen I, Eriksen HM. Influence of coronal restorations on the periapical health of endodontically treated teeth. Endod Dent Traumatol 2000;16(5):218-221.
65. Zakizadeh P, Marshall SJ, Hoover CI, Peters OA, Noblett WC, Gansky SA, et al. A novel approach in assessment of coronal leakage of intraorifice barriers: a saliva leakage and micro-computed tomographic evaluation. Journal Of Endodontics 2008;34(7):871-875.
66. Tselnik M, Baumgartner JC, Marshall JG. Bacterial leakage with mineral trioxide aggregate or a resin-modified glass ionomer used as a coronal barrier. Journal Of Endodontics 2004;30(11):782-784.
67. de Boer IH, Bangalore S, Benetos A, Davis AM, Michos ED, Muntner P, et al. Diabetes and Hypertension: A Position Statement by the American Diabetes Association. Diabetes Care 2017;40(9):1273-1284.
68. Bernick S, Nedelman C. Effect of aging on the human pulp. Journal Of Endodontics 1975;1(3):88-94.
69. Gutarts R, Nusstein J, Reader A, Beck M. In Vivo Debridement Efficacy of Ultrasonic Irrigation Following Hand-Rotary Instrumentation in Human Mandibular Molars. Journal of Endodontics 2005;31(3):166-170.
70. de Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, et al. Treatment outcome in endodontics: the Toronto study--phase 4: initial treatment. Journal Of Endodontics 2008;34(3):258-263.
71. Lee AHC, Cheung GSP, Wong MCM. Long-term outcome of primary non-surgical root canal treatment. Clinical Oral Investigations 2012;16(6):1607-1617.
72. Schulze A, Schönauer M, Busse M. Sudden improvement of insulin sensitivity related to an endodontic treatment. Journal Of Periodontology 2007;78(12):2380-2384.
73. Geerlings SE, Hoepelman AIM. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunology and Medical Microbiology 1999;26:259-265.

74. Delamaire M, Maugendre D, Moreno M, Le Goff MC, Allannic H, Genetet B. Impaired leucocyte functions in diabetic patients. Diabetic Medicine: A Journal Of The British Diabetic Association 1997;14(1):29-34.
75. Iacopino AM. Periodontitis and diabetes interrelationships: role of inflammation. Annals Of Periodontology 2001;6(1):125-137.
76. García-Hernández A, Arzate H, Gil-Chavarría I, Rojo R, Moreno-Fierros L. High glucose concentrations alter the biomineralization process in human osteoblastic cells. Bone 2012;50(1):276-288.
77. Segura-Egea JJ, Castellanos-Cosano L, Machuca G, López-López J, Martín-González J, Velasco-Ortega E, et al. Diabetes mellitus, periapical inflammation and endodontic treatment outcome. Medicina Oral, Patologia Oral Y Cirugia Bucal 2012;17(2):e356-e361.
78. Hu H, Jiang H, Ren H, Hu X, Wang X, Han C. AGEs and chronic subclinical inflammation in diabetes: disorders of immune system. Diabetes/Metabolism Research and Reviews 2015;31(2):127-137.
79. Tanaka K-i, Yamaguchi T, Kaji H, Kanazawa I, Sugimoto T. Advanced glycation end products suppress osteoblastic differentiation of stromal cells by activating endoplasmic reticulum stress. Biochemical and Biophysical Research Communications 2013;438(3):463-467.
80. Rohlin M, Kullendorff B, Ahlqwist M, Henrikson CO, Hollender L, Stenstrom B. Comparison between panoramic and periapical radiography in the diagnosis of periapical bone lesions. Dento maxillo facial radiology 1989;18(4):151-155.
81. Reit C. Decision strategies in endodontics: on the design of a recall program. Endodontics & Dental Traumatology 1987;3(5):233-239.
82. Gutmann JL. Prevalence, location, and patency of accessory canals in the furcation region of permanent molars. Journal Of Periodontology 1978;49(1):21-26.
83. Sim IGB, Lim T-S, Krishnaswamy G, Chen N-N. Clinical Research: Decision Making for Retention of Endodontically Treated Posterior Cracked Teeth: A 5-year Follow-up Study. Journal of Endodontics 2016;42:225-229.
84. Marquis VL, Dao T, Farzaneh M, Abitbol S, Friedman S. Clinical research: Treatment Outcome in Endodontics: The Toronto Study. Phase III: Initial Treatment. Journal of Endodontics 2006;32:299-306.
85. Low KM, Dula K, Burgin W, von Arx T. Comparison of periapical radiography and limited cone-beam tomography in posterior maxillary teeth referred for apical surgery. J Endod 2008;34(5):557-562.
86. Wu M-K, Wesselink PR. Timeliness and effectiveness in the surgical management of persistent post-treatment periapical pathosis. Endodontic Topics 2005;11(1):25.
87. Maia Filho EM, Martins Calisto A, De Jesus Tavarez RR, de Castro Rizzi C, Bezerra Segato RA, Bezerra da Silva LA. Correlation between the Periapical Index and Lesion Volume in Cone-beam Computed Tomography Images. Iranian Endodontic Journal 2018;13(2):155-158.
88. Estrela C, Bueno MR, Azevedo BC, Azevedo JR, Pécora JD. A new periapical index based on cone beam computed tomography. Journal Of Endodontics 2008;34(11):1325-1331.