Title
The association between dental root canal occlusion (seconday dentin formation) and plasma cholesterol levels

Author
Ashouri, Nahal

Publication Date
2004

Peer reviewed|Thesis/dissertation

The Association between Dental Root Canal Occlusion (Secondary Dentin Formation) and Plasma Cholesterol Levels

By

Nahal Ashouri, DDS

THESIS

Submitted in partial satisfaction of the requirements for the degree of
MASTER OF SCIENCE

in

ORAL and CRANIOFACIAL SCIENCES

in the

GRADUATE DIVISION

Of the

UNIVERSITY OF CALIFORNIA

San Francisco

[redacted]

Acknowledgements

This study was made possible by the efforts of many friends, family, and colleagues, to whom I am very grateful.

I am truly grateful to my committee members, Dr. Harold Goodis, Dr. Arnold Kahn, and Dr. Arthur Miller for their guidance, patience, kindness, and friendship in mentoring my thesis. Their insights, knowledge, and expertise were essential in the entire project from experimental design, image acquisition, and analysis, to writing this thesis.

I would like to thank Dr. Chuck Mcculloch and Miss Ann Lazar for their help with my project's statistics, and for my endless questions and confusions about the statistical analysis.

I am greatly indebted to Dr. Raul Garcia and Dr. Elizabeth Krall and the U.S. Department of Veterans Affairs, K24 DE00419 (NIDCR) for allowing me access to the extensive database of radiographs and patient records at the VA in Boston.

I would also like to thank Pourang Jahanshahi for his assistance in data analysis of this study.

Financial support for this project was provided by the UCSF Department of Growth and Development and by Various Donor's Funds (AJK) and is greatly appreciated.

I would also like to thank my co-residents for their insight and suggestions regarding this study.

I would like to thank my brilliant mother, Helen Khalouyan Pivk, for offering me a job as her maid and putting things into perspective whenever I discussed challenges at school with her! ☺

And last, but not least, I would like to express my deepest thanks to my intelligent, loving, and supportive husband, Stephen Leas. I appreciate him not just for his patience and dedication throughout my seemingly endless years in school, but also for his many hours of data-entry into excel and for his contribution to my thesis.

The Association between Dental Root Canal Occlusion (Secondary Dentin Formation) and Plasma Cholesterol Levels

Nahal Ashouri, DDS

Abstract

Elevated Plasma Cholesterol Levels Appear Predictive of a Faster Root Canal Closure; a Postulated Marker of Human Aging.

Introduction and Hypothesis: One of the anatomic, "senescent "changes that occurrs with age that can be monitored non-invasively in humans is the root canal. This structure becomes smaller, on average, in older individuals due to the deposition of secondary dentine, a mineralized tissue that can be visualized in routine, clinical x-rays. The causal mechanism responsible for stimulating secondary dentine formation ("root canal closure") is unknown but one possibility is that it is secondary to the also age-related diminution in the blood vascular supply to the canal. It has been reported that the latter reduction results, at least in part, from atherosclerotic changes in the small arterioles that feed the soft tissues of canal. On the basis of this possibility, we have hypothesized that the rate of root canal closure would be higher in aging individuals with consistently high plasma cholesterol levels (>240 mg/dl), a major risk factor for atherosclerosis, than in comparably aged individuals with low levels of cholesterol (<200 mg/dl). We have tested this hypothesis using material collected from subjects enrolled in the Longitudinal Dental Study. Methods: At enrollment, the subjects, ranging in age from 30-57 yrs, received comprehensive dental examinations including oral x-rays, along with blood chemistry and other medical evaluations. Subsequently, the subjects returned at ~3 year intervals for oral health assessments and x-rays, and repeat blood chemistry measurements. The average subject participated in >6 follow-up visits equivalent to ~20 years of longitudinal

data gathering. Using digitized x-rays of healthy or minimally restored 1st and 2nd bicuspids, blinded measurements were made of the root canal and root diameter at the cemento-enamel junction and at a position 1/4 down the length of the root. These measurements were then converted to ratios to correct for individual differences in tooth size. The data were then analyzed using a mixed regression statistical model with age, cholesterol level and the occurrence of a myocardial infarct (MI) as the principal variables. Results:. As previously reported, on average, the root canal at both the CEJ and 1/4 sites diminished in size with age ($p=<0.0001$). The rate of closure did, however, vary as a function of cholesterol level; the higher the cholesterol level, the faster the rate (p =.~028 at the CEJ; 0.028 at the 1/4 interval). This relationship was evident in both the slope (rate) and intercept at age 65. The rate of closure was not affected by a history of MI. Conclusion: The data indicate that elevated cholesterol levels are predictive of a more rapid rate of root canal closure (secondary dentine formation) in aging men, and indirectly give credence to the hypothesis that the latter process is linked to atherosclerotic changes in arterioles of the dental pulp.

Table of Contents

Title page	i.
Acknowledgements	ii.
Abstract	iv.
Table of Contents	vi.

INTRODUCTION	1
HYPOTHESIS AND SPECIFIC AIMS	7
MATERIALS AND METHODS	8
RESULTS	16
DISCUSSION	22
CLINICAL SIGNIFICANCE	26
REFERENCES	27

The Association between Dental Root Canal Occlusion (Secondary Dentin Formation) and Plasma Cholesterol Levels

Introduction

Throughout life, human anatomic and physiologic changes are influenced by age and disease. These changes include the dentition and their inner-structures, such as the dental pulp and dentin.

The human dental pulp is a loose connective tissue contained within a central cavity surrounded by an avascular hard tissue case. The pulp itself has a rich vascular supply, with blood vessels entering the pulp space via the apical foramen in association with the sensory and sympathetic nerve bundles.[1] Vascular capillaries constitute a predominant part of the blood transport system of the pulp.[1-3]

The dental pulp, like other body tissues, undergoes age-related changes. These alterations include physiological, defensive, and pathological irritant-induced changes which are sometimes difficult to differentiate one from the other in terms of cause.[4, 5] Extensive studies have evaluated the age-related changes of the dental pulp. Some believe that as humans age, pulp size and volume decrease due to the normal process of continuous apposition of secondary dentin; a process that will narrow the root canal space over time.[1, 6, 7]

Although it has never been directly determined, an age-related interference in circulation and innervation may be the first step in dental pulp aging.[1, 5] Other age-related changes in the pulp include degeneration of odontoblasts, decrease in size and number of fibroblasts, blood vessels, and nerves, and increase in cross-linkages and number of mature collagen fibers, lipid infiltration and calcification.[1, 5, 8, 9] Using histological methods, Bodecker was the first to suggest an association between age and secondary dentin formation.[10]

Age related changes in the dental pulp and dentin

Both the hard and soft tissues of the teeth and their attachment apparatus are subject to constant change. This process begins immediately after tooth eruption and continues throughout life. An exact dividing line between changes which are physiological and pathological cannot always be drawn.[9] The dentin of older people is characterized by the continuous narrowing of the lumens of the dentinal tubules with increasing calcification throughout tubule length,[7, 9, 11] reduction in the amount of tubular fluid, and reduced sensitivity.[9]

Historically, the aging process has been recognized as an important determinant of pulp size which decreases in volume via the formation of secondary dentin.[12] Murray et al,[13] found that with increasing patient age, dentin thickness increased in both crown and root spaces, with the deposition of new dentin tissue appearing to be asymmetrical. As a consequence of this age-related increase, the volume of the pulp space lessens macroscopically.[9] This is considered a normal, non-pathological event and occurs mainly in teeth that are free of caries or restorations, and with no detectable periodontal disease (Figure 1).

An association between age and secondary dentin formation, using histological methods, has been established since 1925.[10] Several cross-sectional studies have confirmed this association, but, no longitudinal studies have investigated this relationship.[5, 7, 9, 12, 13]

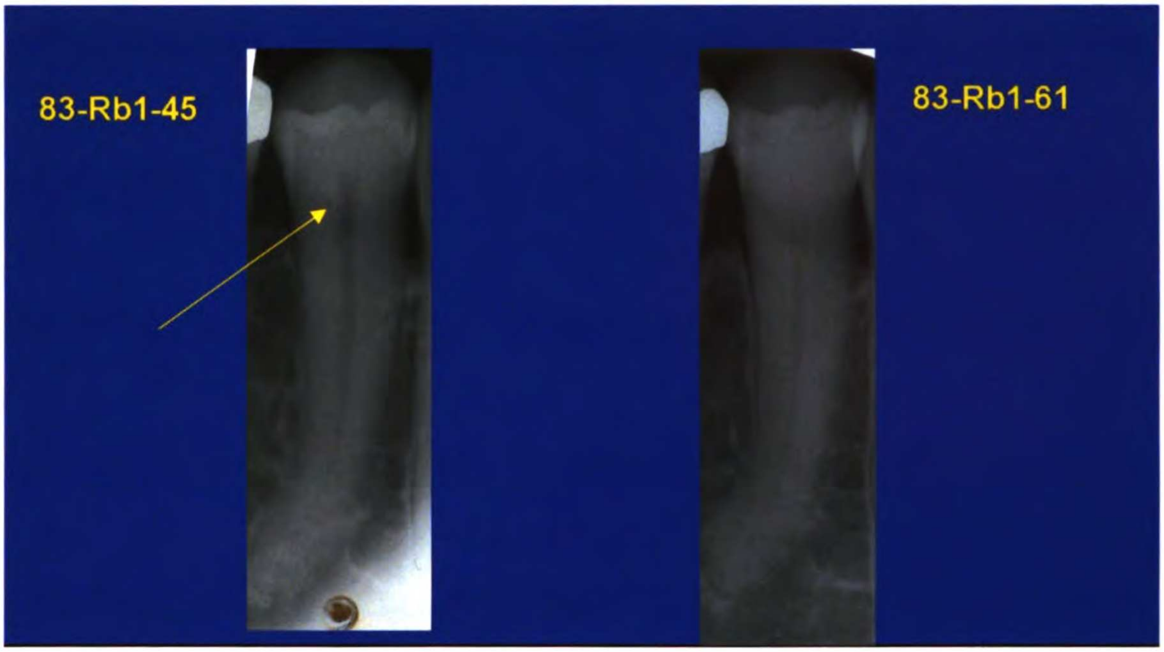

Figure 1. Radiographs showing root canal calcification in a permanent mandibular first bicuspid of a subject with high cholesterol levels (ranging between 235 - 272). Note the patent root canal space in the radiograph on the left (arrow) when the subject was 45 years old and the significant reduction of the canal space at age 61 on the right.

Atherosclerosis

Atherosclerosis is the most common disease in the industrialized world, and by 2020 is predicted to be the number one cause of death worldwide. It is a disease of the intima and media layers of small to medium sized arteries, and develops slowly over many years. A number of risk factors for atherosclerosis have been identified. Most prominent among these factors is an elevated level of plasma cholesterol. The lowering of cholesterol reduces the risk of heart attacks, strokes, and all forms of atherosclerotic vascular disease. [14] There are direct correlations between levels of total plasma cholesterol, high density lipoproteins (HDL), low density lipoproteins (LDL), and coronary artery disease, atherosclerosis, and ischemia to various parts of the body such as the heart, the brain, and other organs.[15]

Trauma and dental root canal system occlusion

Previous studies have shown that reparative dentin formation can be triggered by a large number of traumatic events. These events have been noted to include preparation trauma from a bur or laser, dental operator hand instrumentation, microleakage of bacterial toxins, restorative materials or chemicals, and attrition, abrasion, or erosion.[16-21]

Given the range of trauma that can stimulate root canal system occlusion, it would not be surprising if decreased blood flow to the pulp (ischemia), secondary to atherosclerosis, lead to the same phenomenon. If this is the case, then a causal linkage would exist between an age-related event, loss of vascular supply, and another age-related event, secondary dentin formation (canal occlusion). It would also follow that any circumstance that would increase likelihood of diminished blood flow (elevated cholesterol) would also increase secondary dentin formation.

Correlation between decreased blood flow to the pulp and secondary dentin formation

Arteriosclerosis is a pathological condition in which there is a severe luminal narrowing of arterial vessels, resulting in tissue ischemia and potentially necrosis. Since atherosclerosis also affects the small arterioles of the body, the dental pulp arterioles undergo change as well. [22] Robbins[23] showed that atherosclerotic alterations in pulpal arteries result in a narrowing of vessel lumens and calcification of the vessel walls. As the degree of narrowing increases, circulation to the soft tissues of the pulp becomes more limited, and the tissue becomes more fibrotic changes and may even calcify.[23] Atherosclerosis occurs in arteries in different parts of the body and while more common in older individuals, can almost always be observed in the arteries of young adults. Arteriosclerosis has been reported in the dental pulp of people as young as 40 years of age.[22] Atherosclerotic alterations in pulpal arteries result in a narrowing of vessel lumens and calcification of the vessel walls.[23] These results are consistent with related findings showing that the hemodynamics (blood flow) in the human pulp is reduced with age.[11]

Arteriolar sclerosis is usually restricted to small peripheral arterioles with a diameter of 100μm or less. Considering that the dental pulp consists of arterioles that are equal or less than 50 μm in diameter,[24] it seems likely that these vessels will be affected by vascular ischemia. [5]

Atherosclerotic changes include calcification of the vessel walls, intimal thickening, and elastic hyperplasia, all of which decrease blood supply to the coronal portion of the pulp.[5] It is in the coronal portion of the pulp that secondary dentin formation begins. Morse et.al.,[7] found that pulp vessels in aging individuals undergo atherosclerotic changes that diminish the tissue's blood supply.

Burke et al.,[25] reported that circulatory problems may be one of the initiating factors for an increase in pulpal calcification. There also appears to be a relationship between atherosclerosis and calcification of the soft tissues of the pulp (or formation of pulp stones). In a cross sectional study, Moura et al.,[26] investigated the occurrence of free pulpal calcifications (denticles) in the pulp chamber of teeth of patients suffering from coronary atherosclerosis. The incidence of denticles was higher in patients with coronary atherosclerosis than in patients who did not suffer from this disease. Moreover, patients suffering from coronary atherosclerosis demonstrated a higher number of teeth with pulpal calcification than patients in the control group. [26]

The correlation between a variety of systemic diseases and root canal space occlusion has been previously investigated. Howe found that pulpal stones are signs of an alteration in the patient's general health, and their occurrence in the tooth pulp indicate a correlation to calcified formations in the gall bladder.[27] Several other studies have reported a statistically significant correlation between root canal system closure and calcification related diseases such as arthritis, gout, kidney stones, and atherosclerosis.[28-30]

A comparison of relative dental pulp size in patients with chronic renal disease to healthy controls, by Galili et al.,[31] revealed a significant pulp narrowing in the kidney disease group. A strong correlation between the chronicity of systemic disease and pulp narrowing was found in the premolar and molar teeth of the test group, which indicates the relationship between systemic calcification and dental pulp calcification.[31-34]

While the above studies suggest a link between atherosclerosis, heart disease, and root canal calcification, the specifics of this possible association remain unknown, in part because the earlier observations were based on cross-sectional analysis. Cross-sectional studies mask individual-to-individual variations, making it difficult to clearly assess whether or not root canal calcification is linked to or associated with some exogenous factor or factors. In the present study, we explore the relationship between the rate and/or degree of root canal system closure and plasma cholesterol levels in a longitudinal, retrospective, case-controlled study that extended over many years. Plasma cholesterol levels are used as likely surrogates of atherosclerosis since it was not possible to directly measure atherosclerosis in the teeth of the subjects.

Hypothesis

The hypothesis of this study is that individuals with elevated cholesterol levels will either show an earlier onset of canal closure, or a more rapid rate of canal closure.

This hypothesis will be tested using a case-control, age-adjusted research design comparing the rate of root canal system closure of individuals with high baseline levels of total plasma cholesterol (as defined by the National Cholesterol Education Program) to those with clinically desirable levels of cholesterol.

Materials and Methods

Subjects

The Dental Longitudinal Study (DLS), as a component of the Normative Aging Study, was initiated in 1969 at the Veterans Administration Hospital now associated with Boston University, and is ongoing. The DLS data collection includes serial dental radiographs (periapical, bitewing, and panoramic radiographs) taken at approximately 3 year intervals of entirely male, mostly Caucasian subjects. At baseline , the subjects ranged in age from 25 to 70 years. All enrollees were generally healthy men with two or more teeth. Initially, a total of 804 dentate individuals were enrolled in the study. The current number is about 700 males, some of whom have been followed for more than 30 years. In addition to the comprehensive dental examinations, the subjects also received extensive medical assessments including medical histories and physical, physiological, and psychological examinations.[35-38] The physical measurements included plasma cholesterol, HDL and triglyceride levels, and notations of myocardial infarction and smoking.

Sample size and description of cohort

A total of 65 adult male subjects and 125 teeth were included in this study. The median number of visits per subject was four, with a range of 3-11 visits. Two groups of subjects were defined by their cholesterol levels. Initially, 27 of the subjects were in the low cholesterol group (below 200 mg/dl), and 38 were in the high cholesterol group (above 240 mg/dl), as defined by the National Cholesterol Education Program in 2001 (Table 1).[39] Only subjects with stable cholesterol levels over time were selected (Figure 2).

Calssification of LDL, Total, and HDL Cholesterol (mg / dl)	
LDL Cholesterol	
< 100	Optimal
100-129	Near or above optimal
130-159	Borderline high
160-189	High
>190	Very high
Total Cholesterol	
< 200	Desireable
200-239	Borderline High
>240	High
HDL Cholesterol	
<40	Low
>60	High

Table 1. Classification of LDL, Total, and HDL cholesterol (mg/dl)
For the experimental group, subjects with consistently high total plasma cholesterol levels from the start of the study, were selected. Similarly, for the control group, subjects with consistently low total plasma cholesterol levels were selected.

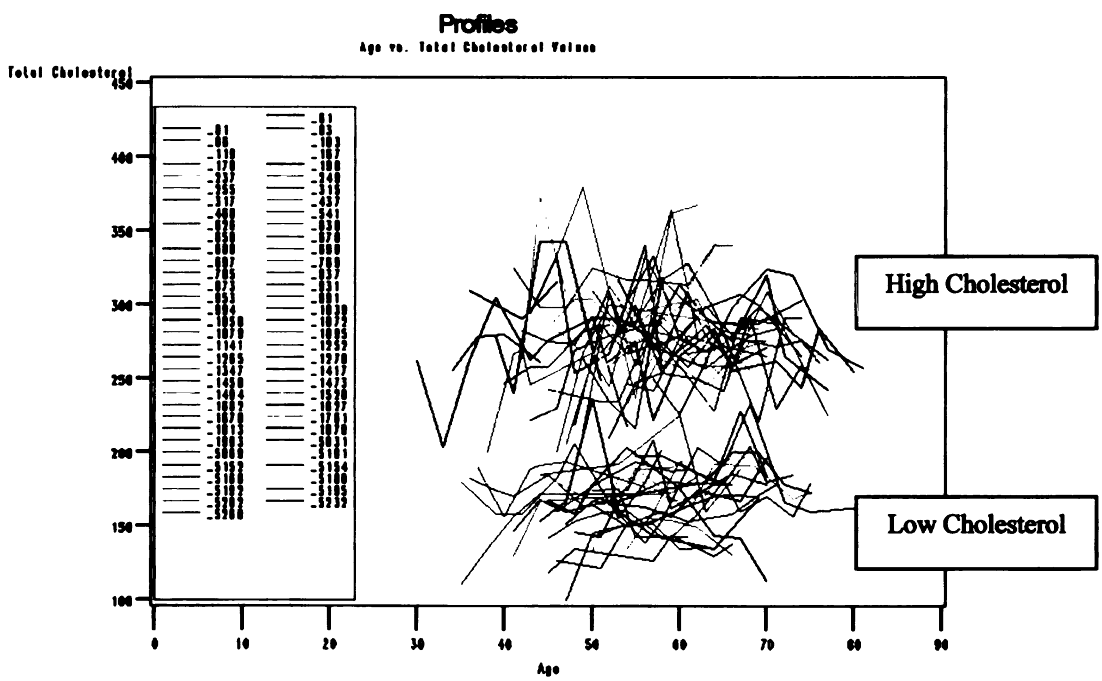

Figure 2. Age versus Total Cholesterol values are shown for every subject at every time point. Note that the total cholesterol value for each subject is stable over time, staying within defined boundaries of "high cholesterol" and "low cholesterol" groups.

At the time of initial radiograph, the subjects ranged from 30 to 57 years old (mean = 46.83 +/- 6.65 years). At the time of the final radiographs, the subjects ranged from 43 to 81 years old (mean = 66.98 +/- 8.84 years, Table 2). The distribution of mean ages and standard deviations for both the high and low cholesterol groups at the initial and final recordings are shown in Figure 3.

In addition to blood tests and complete medical exams, notations were made in each patient's chart after each visit regarding smoking and myocardial infarction incidents. As shown in Table 2, 48% of the low cholesterol subjects, and 58% of the high cholesterol subjects were smokers at some time in their lives, which was almost always before the last set of data collected on the patient. 11% of the low cholesterol subjects and 13% of the high cholesterol subjects experienced episodes of myocardial infarction.

Subjects	Low Cholesterol group (below 200 mg / dl)		High Cholesterol Group (Above 240 mg / dl)	
	Initial	Final	Initial	Final
Average age (years)	47.54	67.61	47.02	66.56
Standard deviation	6.69	7.98	6.69	9.44
Minimum	34	43	30	44
Maximum	57	80	57	81
% Smoking	48% (13/27)		58% (22/38)	
% MI incidence	11% (3/27)		13% (5/38)	

Table 2. Subject age distribution at the initial and final data collection, as well as percentage of subjects smoking and myocardial infarct incidences in each cholesterol group. Note that the two groups have similar means and standard deviations of age at the initial and final stage of data collection.

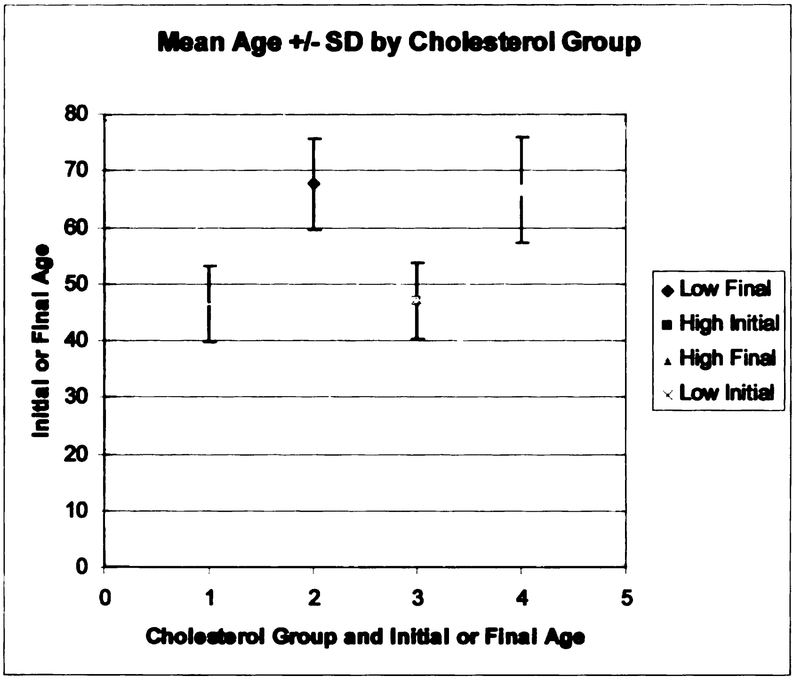

Figure 3. Mean ages and standard deviations for the experimental and control groups at the initial and final data recording. Note that the two groups have similar means and standard deviations of age at their initial and final time of data recording.

Study Design & Case Selection

A case control experimental design was used for this study. With previous literature as a guide for subject selection,[39] an age-matched experimental group and a control group were selected from historical records obtained from the VA Longitudinal and Normative Aging Study based on baseline plasma total cholesterol levels. The latter measurements were used as our main initial selection criteria, since HDL and LDL values were not available during the initial years of the study. However, HDL values were used for data analysis once they became available. Table 1, from the third report of the National Cholesterol Education Program[39], was used to establish the cut-off values for inclusion in the control vs. experimental groups.

Our initial approach of having only one low and one high cholesterol group proved uninformative, so in subsequent analysis, we placed the subjects into 5 groups based on their total cholesterol levels, as followed:

1. below 170 mg / dl
2. between 170 and 200 mg / dl
3. between 200 and 240 mg / dl
4. between 240 and 280 mg / dl
5. above 280 mg / dl

Periapical radiograph inclusion criteria

We studied radiographically healthy right and left mandibular first and second bicuspids. The presence of occlusal restorations with less than 1 mm penetration into enamel was deemed acceptable.

Periapical radiograph exclusion criteria

Teeth with pulp stones, root canal treatment, more than one root canal system or carious lesions past the DEJ, were excluded. Also teeth with root caries, fractures into dentin, more than one surface restoration, or surface restorations penetrating more than 1 mm into dentin, as viewed by bitewing or periapical radiographs, were not used. Finally, teeth with cervical restorations, the presence of a crown or bridge, incomplete images of the tooth, or poor quality radiographs, were also excluded.

Radiographic Analysis

Each periapical radiograph was examined for inclusion and exclusion criteria. The age of the subject at the time each radiograph taken was recorded. Radiographs were scanned at 1000 dpi as a color image and saved in the JPEG format. The digital images were then cropped, and rotated so that the long axis of the tooth was parallel to the vertical using Adobe Photoshop 6.0 (Adobe, San Jose, California). All images were given a number to achieve operator blindness. Images were then measured using Photoshop 6.0 software(Adobe, San Jose, California).

To make the measurements at defined and reproducible sites on the root, a line was drawn horizontally, passing through the cemento-enamel junction (CEJ). Then, a vertical line was drawn perpendicular to this line and extended from the cement-enamel junction (CEJ) to the apex of the root. This line was divided into four equal quarters (Figure 4). Measurements were made of the dimension of the root and the root canal at three sites; the CEJ, at the ¼ point below the CEJ, and along the axis of the root. From these measurements, three ratios were calculated; the CEJ ratio, the ¼ ratio, and the Height ratio.

The CEJ ratio was defined as the ratio of the width of the root canal space to the width of the root at the CEJ. The ¼ ratio was the ratio of the width of the root canal space to the width of the root at the ¼ increment. The Height ratiowas the ratio of the length of the most occlusal portion of the root canal space to the apex over the length of the root (from the CEJ to the apex). The ½ and ¾ locations were used to verify whether the root canal space was open or closed at those locations (Figure 4). In addition to the above, medical records were examined to determine if and when the subjects had a history of myocardial infarction and smoking. The latter findings were recorded as binary date, 1=yes and 0=No.

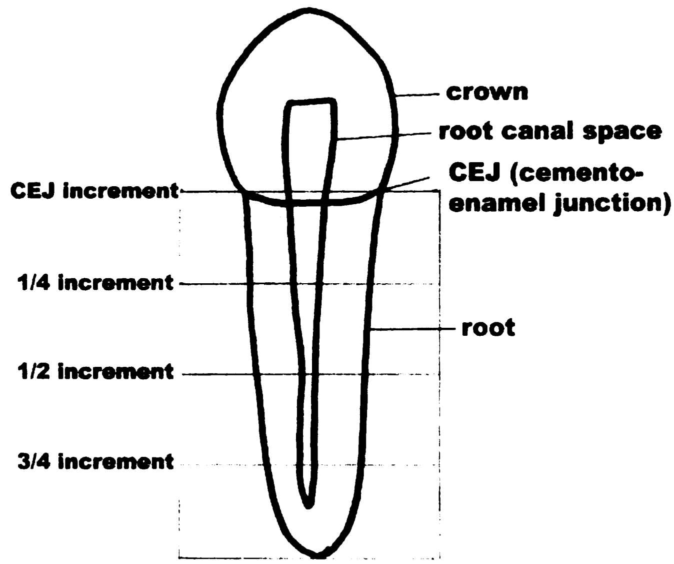

FIGURE 4. Schematic of tooth and areas of measurement

Intra-operator & Inter-operator Error

Ten images were selected at random, and duplicate measurements were made by each of two examiners to determine intra-operator error. Both operators measured all variables for all 10 teeth for inter-operator calculations. Coefficient of variation was calculated for each error using the following equation:

$$\text{Coefficient of variability} = \frac{[\text{mean square (residual)}]^{1/2}}{\text{Mean of all data}} \times 100\% = \frac{\text{Standard deviation}}{\text{Average}} \times 100\%$$

The inter-operator and intra-operator errors were calculated. The coefficient of variation was about 2% for operator 1 and 6% for operator 2. The operator 2 standard deviation values were higher than operator 1 values (SD 3 or 4 times larger). The reasons for this were not clear. However, and importantly, both operators' calculated similar CEJ ratio and quarter ratios with no statistical significance. There were, however, statistically significant differences in determining the canal length ratios. This difference appears to be due to the difficulty in recognizing the most occlusal border between the hard tissue of the dentin and the soft tissue of the pulp, within the crown, in a two-dimensional x-ray of a three-dimensional tooth. The length ratios made by both operators were generally inconsistent, and therefore showed no significant trend.

Statistical Analysis/ Calculations

The CEJ, ¼, and Height ratios were calculated for each of the multiple measurements made on the subjects. These data were then used to determine whether there was a statistically significant association between rate of root canal closure and age and, if so, whether this association was affected by total plasma cholesterol levels.

A mixed regression model was used to accommodate the repeated measurements of teeth and subjects for each of the three outcomes: CEJ ratio, ¼ ratio, and Height ratio. In each model, adjustments were made for tooth type (i.e., left or right, first or second premolars). In addition, the effects of myocardial infarction, HDL, smoking, and age were investigated. SAS version 8.2 statistics software (SAS Institute, Cary NC) was used for the statistical analysis.

An alternating logistic regression[40] was used for modeling patency (canal open or closed) at ½ and ¾ lengths of the root in order to accommodate the repeated measurements over teeth and subjects. As before, adjustments were done for the predictors of myocardial infarction, HDL, smoking, and age. In addition, residual plots were analyzed to assess whether the rates of closure were normally distributed over time. The residual plots were also evaluated to assess and identify potential outliers.

Results

Cholesterol Levels

A summary of the p-values for the relationship of canal space closure and age, mean change per year in ratio, and 95% confidence intervals for the CEJ, ¼ ratio, and height ratios and P-values of the ½ and ¾ increments are shown in Table 3.

Increment	P-value	Mean	Lower confidence level	Upper confidence level
CEJ Ratio	<0.0001 *	-0.0285	-0.0326	0.0243
¼ Ratio	<0.0001 *	-0.0107	-0.0148	0.0066
Height Ratio	0.0976	-0.0068	-0.0148	0.0012
½ Increment	0.4693			
¾ Increment	0.5734			

Table 3. P-value, mean change per decade in the ratio, and 95% confidence intervals for each increment. (* = statistically significant)

As shown in Table 3, there is a statistically significant diminution in root canal size as a function of age at the CEJ and ¼ ratios. The change in height shows the same trend but does not reach statistical significance in this study; perhaps because of the degree of inter-operator difference in height measurements and the difficulty in accurately measuring this dimension.

The potential impact of cholesterol level on rate of canal closure using continuous outcomes is shown in Figures 5, 6, 7, and 8. These data have been adjusted for myocardial infarct, HDL, and smoking. In Figure 5, a more rapid rate of decrease of CEJ ratio is found in the higher cholesterol groups and is statistically significant. The confidence interval is -.0002 to 0.000 (p = 0.0258).

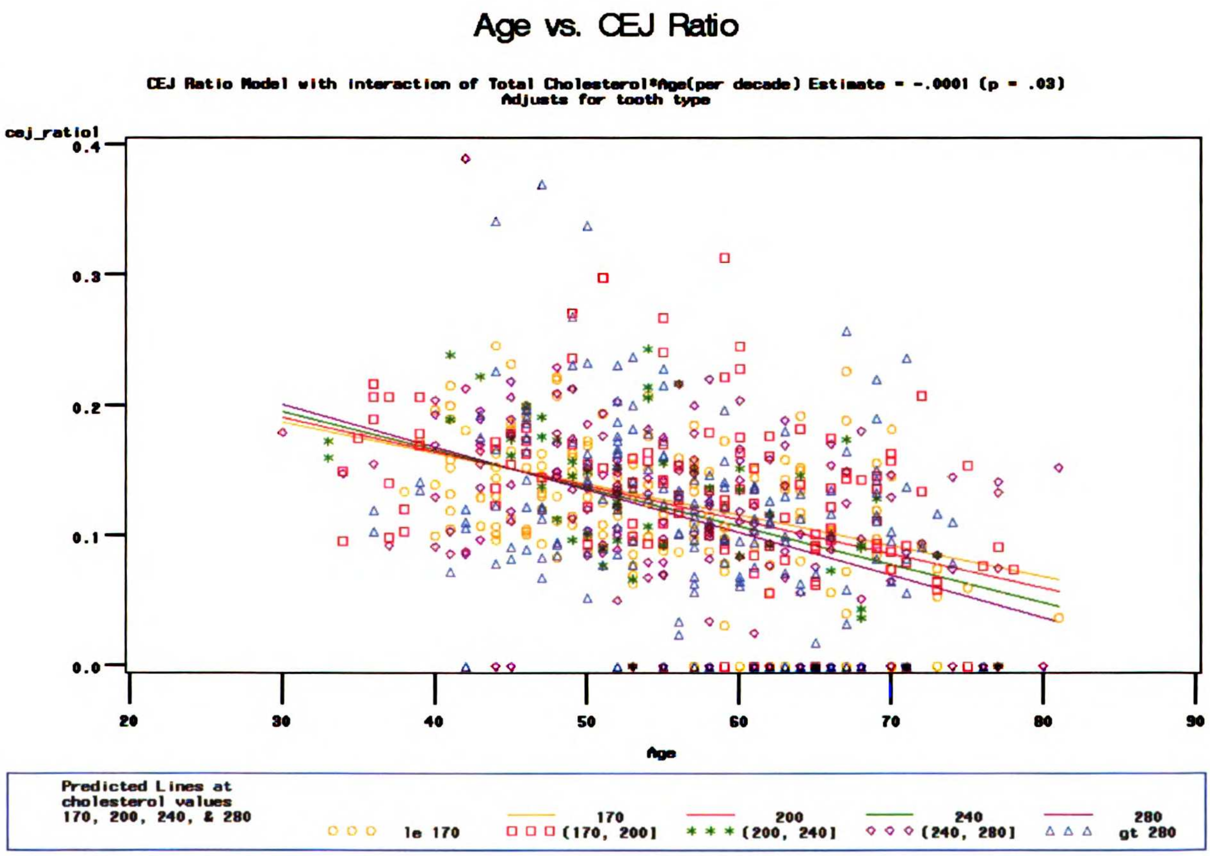

Figure 5. Change in CEJ ratio over time. Note the steeper rate of decrease of the CEJ ratio (slope) as cholesterol levels increase, indicating a more rapid rate of closure of the root canal system at the CEJ level.

To highlight the differences in CEJ ratio of the various cholesterol groups the intercept at subject age 65 of all these lines was plotted in a bar graph (Figure 6). Note the apparent stepwise progression from lower to higher cholesterol levels; lower cholesterol levels equating to more open canals at the CEJ.

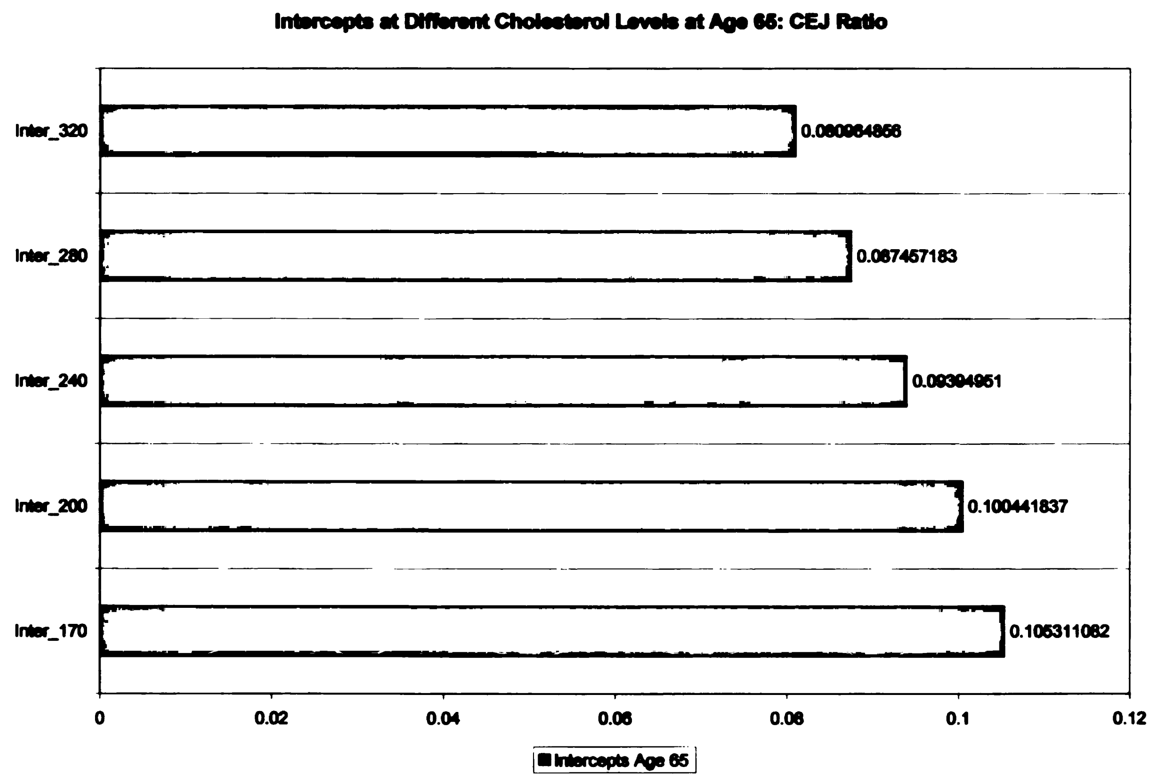

Figure 6. Intercepts of the CEJ ratio of different cholesterol levels at age 65, derived from Figure 5. Note the larger CEJ ratio at lower cholesterol levels, indicating a smaller root canal ratio at the CEJ for the subjects in the higher cholesterol groups.

As shown in Figures 7 and 8, a similar phenomenon regarding the rate of canal closure and cholesterol level is seem at the ¼ site (ratio). After adjustment for MI, HDL, and smoking, elevated cholesterol is associated with an incrementally more rapid rate of canal closure (Figure 7, p = 0.0260; CI= -.0001 to 0.000). These increments become more apparent when the intercept at age 65 is plotted against cholesterol level (Figure 8).

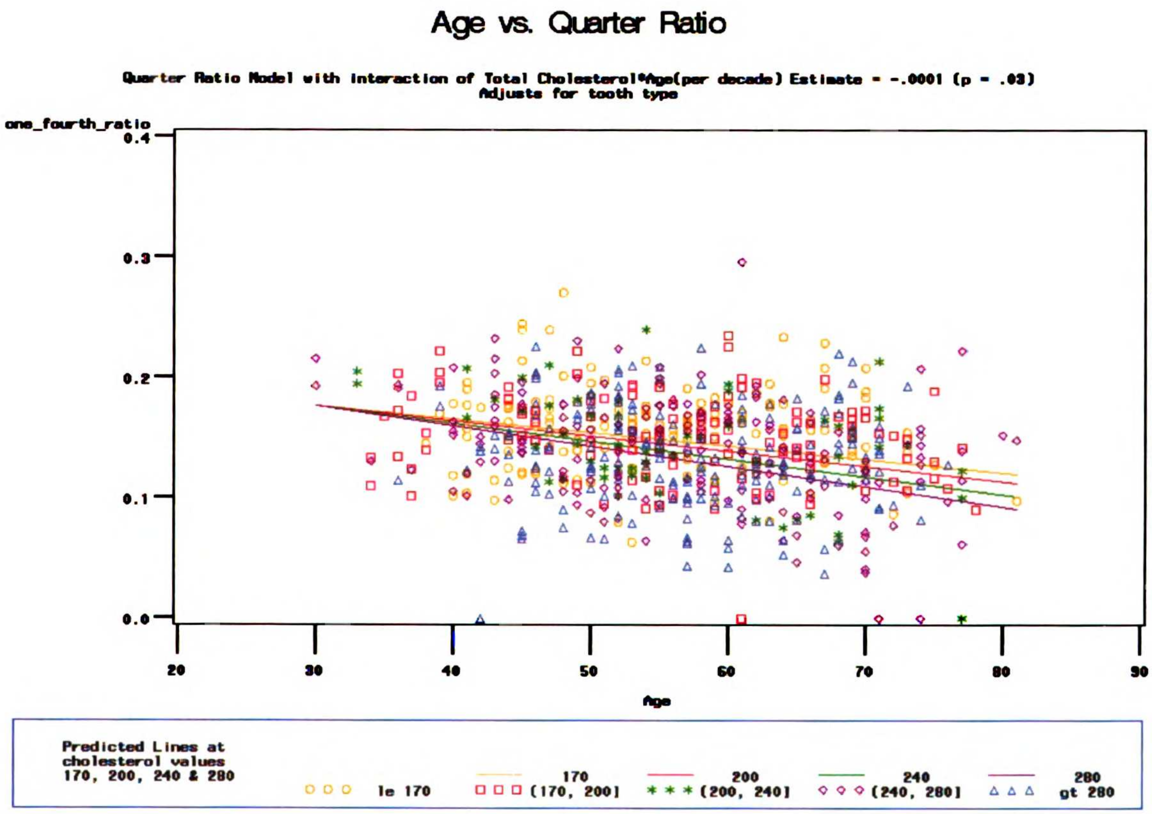

Figure 7. Change in ¼ ratio over time. Again, a steeper rate of decrease of the ¼ ratio (slope) was observed as cholesterol levels increase, indicating a more rapid rate of closure of the root canal system at the ¼ level.

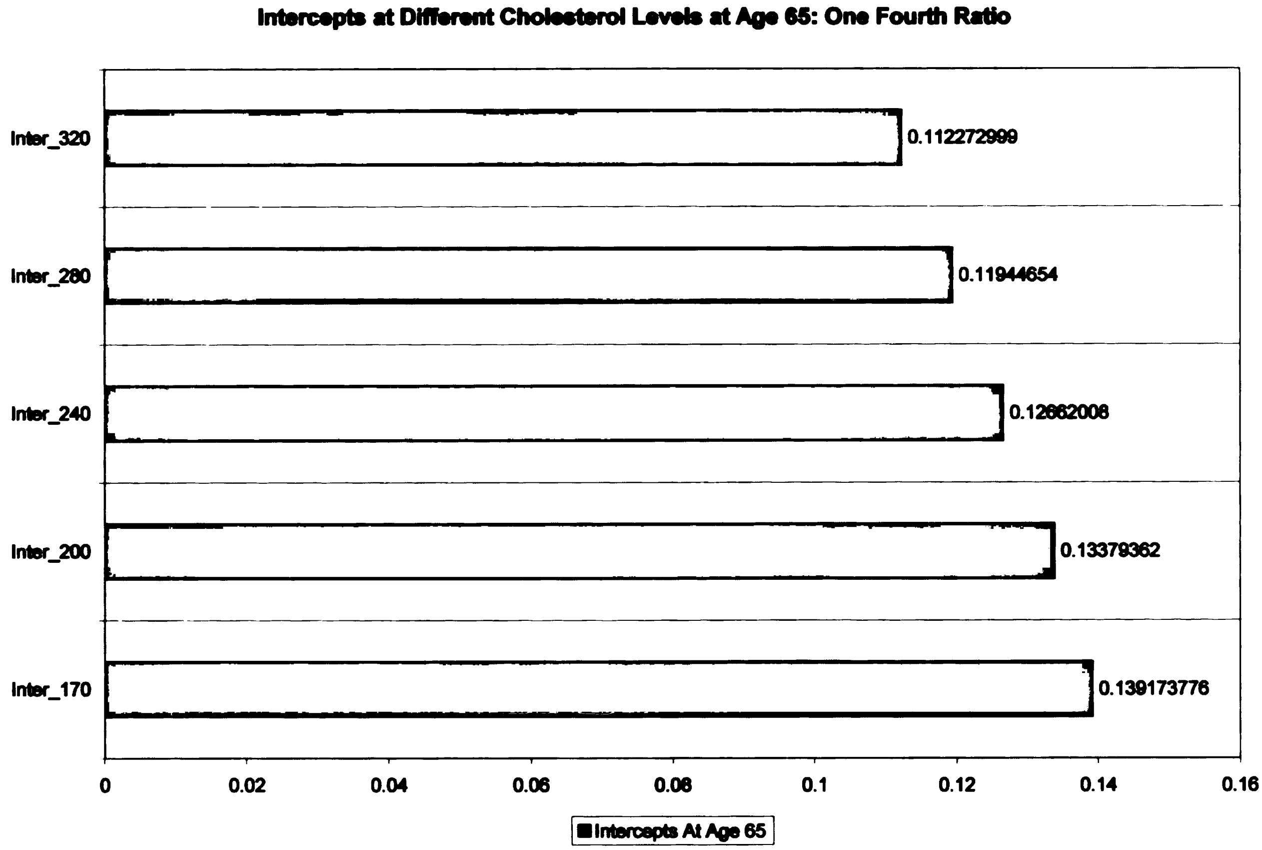

Figure 8. Intercepts of the 1/4 ratio of different cholesterol levels at age 65, derived from Figure 7. Note the larger 1/4 ratio at lower cholesterol levels, indicating a smaller root canal ratio at the 1/4 for the subjects in the higher cholesterol groups.

In contrast to the findings at the CEJ and ¼ ratios, changes in height ratio (adjusted for myocardial infarction, HDL, smoking, and age) with age were not significantly altered by plasma cholesterol levels (data not shown). Similarly, using alternating logistic regression analysis, no association was found between cholesterol levels and canal patency at the ½ and ¾ levels (data not shown).

Myocardial Infarct

The mixed regression model of the CEJ ratio, adjusted for cholesterol levels, shows that the rate of decrease in the CEJ ratio as a function of age is similar for those subjects who did and did not experience an MI. However, those who experienced myocardial infarcts may have larger canals at the CEJ initially, and as they age (Figure 9). This latter difference, however, is not statistically significant (p = 0.0830), but suggests a possible trend. The data at the ¼ ratio show a similar trend, but again, the data do not establish statistical significance (results not shown).

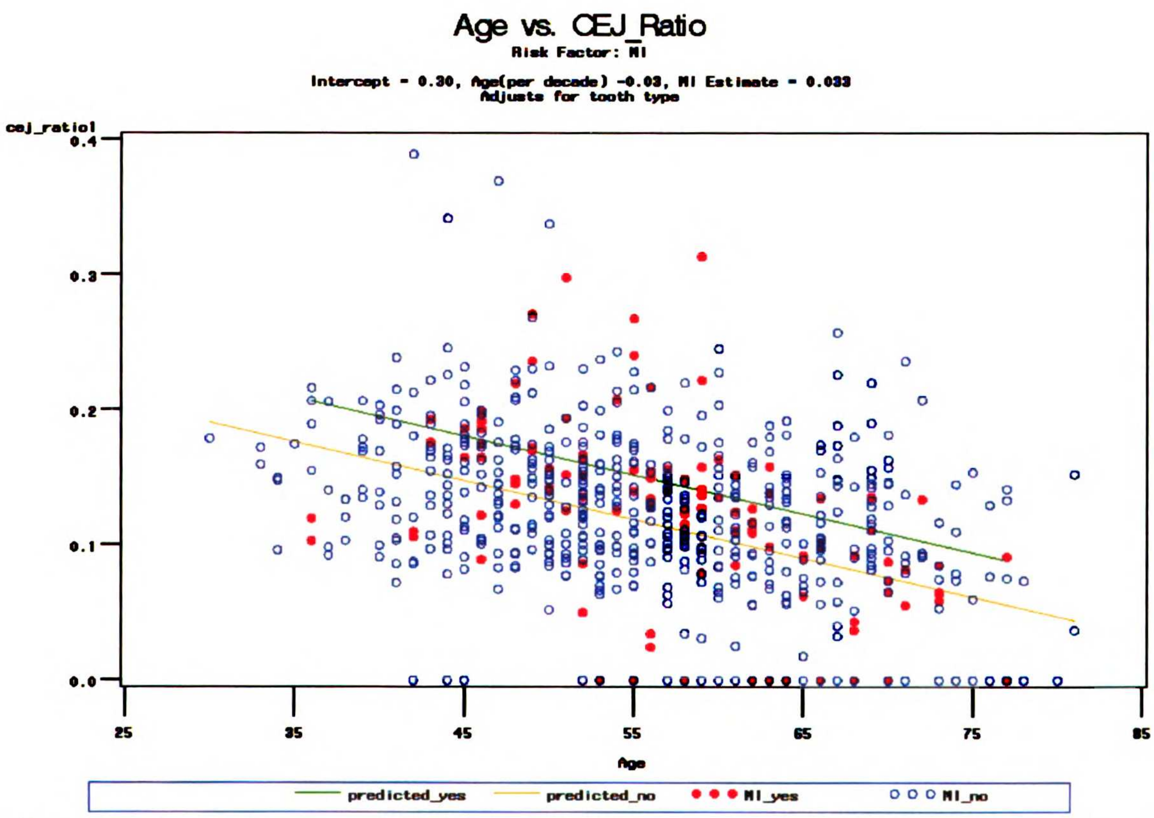

Figure 9. Change in CEJ ratio over time. After adjustment for cholesterol levels, rate of decrease in CEJ ratio for both groups were similar. Note that those who experienced myocardial infarcts may have had a higher CEJ ratio value overall (not statistically significant).

Discussion

Vascular occlusion due to atherosclerotic changes in pulp arterioles may be associated with (and perhaps causes) secondary dentin formation. Since it is not possible to directly measure atherosclerotic change in teeth of living individuals, elevated cholesterol, a major risk factor for atherosclerosis, was used as a surrogate in vessel occlusion. This study was carried out to determine whether plasma cholesterol levels affect the relationship between the rate of root canal closure (secondary dentin formation) and age. In addition to evaluating this relationship, the possible association between the rate of root canal closure and myocardial infarction, HDL cholesterol levels, smoking, and age was also investigated.

Atherosclerosis and secondary dentin formation

There are direct correlations between levels of total plasma cholesterol, and coronary artery disease, atherosclerosis, and ischemia to various parts of the body including the heart, the brain, and other organs. The most prominent risk factor for atherosclerosis has been identified as elevated level of plasma cholesterol. The lowering of cholesterol reduces the risk of heart attacks, strokes, and all forms of atherosclerotic vascular disease.[14, 15]

The relationship between blood vascular supply, calcium homeostasis and dental pulp calcification has been suggested in previous studies. For example, a strong association between chronic renal disease and pulp narrowing was reported by Galili.[31-34] Burke et al., found that reduction of blood circulation may be one of the initiating factors for an increase in secondary dentin formation.[25] Similarly, Moura et al., showed that patients suffering from coronary atherosclerosis had a higher number of teeth with secondary dentin formation than patients in the control group without sclerotic change. [26]

Although these earlier studies indicate a link between atherosclerosis, heart disease, and root canal calcification, the specifics of this possible association remained unknown, mainly because the earlier observations were based on cross-sectional analysis. Cross-sectional studies can disguise individual-to-individual variations, making it challenging to clearly assess whether or not root canal calcification is linked to or associated with some exogenous factor or factors. In this study, we investigated the relationship between the rate and/or degree of root canal closure and plasma cholesterol levels in a longitudinal, retrospective, case-controlled study that extended over many years.

Since it is impossible to directly determine the degree of secondary dentin formation and/or amorphous calcification in living teeth, the occurrence of both processes were assessed in radiographs of teeth as the loss of translucence to x-ray and increasing opacity of the root canal system.[5, 7, 9, 12]

Using radiographs and medical records collected from a subset of 65 subjects enrolled in the Dental Longitudinal Study, we investigated whether elevated cholesterol levels would increase the rate or amount of root canal occlusion. Twenty seven of these individuals had consistently low total plasma cholesterol levels (defined by the National Cholesterol Education Program)[39] over time. The remaining thirty eight subjects had consistently high total plasma cholesterol levels over time.

Three radiographic indices of canal occlusion at the CEJ, 1/4 ratio, and Height ratio, were used to assess the relationship between rate of canal closure and total plasma cholesterol levels. A more rapid rate of decrease of CEJ ratio was found in the higher cholesterol groups and was statistically significant ($p = 0.0258$). Similarly, a higher rate of decrease of the 1/4 ratio was found in the higher cholesterol groups, and was statistically significant ($p = 0.0260$). In contrast, no association was found between the rate of change of the height ratio and plasma cholesterol levels ($p > 0.05$).

Association between canal space occlusion and other systemic factors (myocardial infarction, HDL, smoking, and age)

Medical and dental records of the studied subjects were examined to determine if any relationship existed between root canal closure and myocardial infarction, root canal closure and HDL, or root canal closure, smoking, and age.

In contrast to the apparent association between the degree of closure and total plasma cholesterol level, the consequences of having a history of MI are somewhat less certain. The rate of decrease in the CEJ ratio for subjects who had myocardial infarcts, and those who did not have myocardial infarcts were similar. However, those who experienced myocardial infarcts, showed a higher CEJ ratio value overall. This effect however was borderline and was not statistically significant ($p = 0.0830$). Our finding of higher CEJ ratio in the subjects who had myocardial infarct is contrary to a recent study of this same relationship. Khosrovani et al., showed that the subjects who had an incidence of myocardial infarct had smaller CEJ ratios compared to the subjects who did not have a history of MI.[41] This difference in results could be attributed to the difference in our sample selection method or because of the small sample size. However, it does appear that the presence of atherosclerosis acts to increase closure early in adult life and once established, remains a constant factor in canal occlusion.

Previous cross sectional studies have shown that, on average, pulp size and volume decrease with age. [1, 6, 7, 9, 11-13, 42] The results of the present retrospective longitudinal study also show that, overall, the closure of the canal progresses steadily as individuals become older, thus confirming the findings of previous studies. There were clear individual variations among subjects, but this trend of decreasing root canal width with age was not only present, but also statistically significant (P-value for both the CEJ and ¼ ratios were < 0.0001).

No significant associations were observed between canal occlusion and plasma HDL values. However, HDL values were not measured until recently, there weren't sufficient data available with HDL values for a reasonable analysis. In addition, as shown in a prior study[42], no significant associations were observed between canal occlusion and smoking.

Limitations and Future Possibilities

Even though this study involved more subjects that similar past studies,[41, 42] the size of the cohort was still relatively small, and further limited by the fact that the subjects were all male. Having a larger pool of subjects might well improve the strength of our outcome and clarify the situation with regard to a possible effect of MI. In addition, even if the present results prove valid in confirmatory studies using DLS subjects, having access to radiographs and medical records of women and men of different ethnic groups would allow determination of the general applicability of the findings.

Probably, the greatest limitation to the current technique was the use of indirect measures of both atherosclerosis and secondary dentin formation. Total plasma cholesterol levels were used as surrogates of atherosclerosis, and radiographic images to assess secondary dentin formation (root canal calcification). In the future, if new technology allows for measurement of these variables directly, the validity of this study can be improved.

Finally, root canals are three dimensional structures, which are reduced to two-dimensional radiographs. Radiographic superimposition of structures and marked changes in canal morphology such as splitting of canals can create anomalous findings.[43] Moreover, height ratio proved to be a challenge to measure due to overlapping structures present in the radiographs. This complication contributed to the difficulty in identification of the most occlusal portion of the canal during our measurements. Due to the superimpositions of hard and soft tissue on a radiograph, it was at times very difficult to accurately mark, and therefore measure, the boundary between soft tissue of pulp and the mineralized tissue of dentin that forms the wall of the root canal.

In the present study, the possible effect of cholesterol controlling medications such as lipitor and other statins on canal closure was not been investigated. These medications were not available until the latter part of our subjects' data collection phase and, in any case, our subjects were selected because their cholesterol levels changes little over the years.

Clinical Significance

If there is a protocol in place for the correlation between the rate or amount of root canal closure in teeth and cardiovascular disease, dental radiographs may serve as a screening tool to identify patients who are at risk for such diseases. This is crucial since dental radiographs are taken much more often than blood tests are done.

References

1. Espina AI, Castellanos AV, Fereira JL. Age-related changes in blood capillary endothelium of human dental pulp: an ultrastructural study. Int Endod J 2003;36(6):395-403.
2. Matthews B, Andrew D. Microvascular architecture and exchange in teeth. Microcirc 1995;2(4):305-13.
3. Vongsavan N, Matthews B. The vascularity of dental pulp in cats. J Dent Res 1992;71(12):1913-5.
4. Quigley MB. Functional and geriatric changes of the human pulp. Oral Surg Oral Med Oral Pathol 1971;32(5):795-806.
5. Morse DR. Age-related changes of the dental pulp complex and their relationship to systemic aging. Oral Surg Oral Med Oral Pathol 1991;72(6):721-45.
6. Solheim T. Amount of secondary dentin as an indicator of age. Scand J Dent Res 1992;100(4):193-9.
7. Morse DR, Esposito JV, Schoor RS. A radiographic study of aging changes of the dental pulp and dentin in normal teeth. Quint Int 1993;24(5):329-33.
8. Saad AY. Regressive changes of the dental pulp complex in retained primary molars with congenitally missing successor teeth. J Clin Pediatr Dent 1997;22(1):63-7.
9. Ketterl W. Age-induced changes in the teeth and their attachment apparatus. Int Dent J 1983;33(3):262-71.
10. Bodecker. A consideration of some of the changes in teeth from young to old age. Dental Cosmos 1925;67:106-37.
11. Ikawa M, Komatsu H, Ikawa K, Mayanagi H, Shimauchi H. Age-related changes in the human pulpal blood flow measured by laser Doppler flowmetry. Dent Traumatol 2003;19(1):36-40.
12. Woods MA, Robinson QC, Harris EF. Age-progressive changes in pulp widths and root lengths during adulthood: A study of American blacks and whites. Gerodontology 1990;9(2):41-50.
13. Murray PE, Stanley HR, Matthews JB, Sloan AJ, Smith AJ. Age-related odontometric changes of human teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93(4):474-82.
14. Scott J. The pathogenesis of atherosclerosis and new opportunities for treatment and prevention. J Neural Transm Suppl 2002(63):1-17.
15. Castelli WPW, P.W.F.; Levy, D; and Anderson, K. Serum lipids and risk of coronary artery disease. Atherosclerosis Reviews 1990;21:7-20.
16. Takamori K. A histopathological and immunohistochemical study of dental pulp and pulpal nerve fibers in rats after the cavity preparation using Er:YAG laser. J Endod 2000;26(2):95-9.
17. Magloire H, Romeas A, Melin M, Couble ML, Bleicher F, Farges JC. Molecular regulation of odontoblast activity under dentin injury. Adv Dent Res 2001;15:46-50.
18. Bjorndal L. Presence or absence of tertiary dentinogenesis in relation to caries progression. Adv Dent Res 2001;15:80-3.

19. Piattelli A, Trisi P. Pulp obliteration: A histological study. J Endod 1993;19(5):252-4.
20. Cox CF, White KC, Ramus DL, Farmer JB, Snuggs HM. Reparative dentin: Factors affecting its deposition. Quintessence Int 1992;23(4):257-70.
21. Stanley HR, Pereira JC, Spiegel E, Broom C, Schultz M. The detection and prevalence of reactive and physiologic sclerotic dentin, reparative dentin and dead tracts beneath various types of dental lesions according to tooth surface and age. J Oral Pathol 1983;12(4):257-89.
22. Krell KV, McMurtrey LG, Walton RE. Vasculature of the dental pulp of atherosclerotic monkeys: light and electron microscopic findings. J Endod 1994;20(10):469-73.
23. Robbins SM. Pulpal pathosis secondary to systemic cardiovascular disease. J Endod 1980;6(7):650-1.
24. Ekblom A, Hansson P. A thin-section and freeze-fracture study of the pulp blood vessels in feline and human teeth. Arch Oral Biol 1984;29(6):413-24.
25. Burke FM, Samarawickrama DY. Progressive changes in the pulpo-dentinal complex and their clinical consequences. Gerodont 1995;12(12):57-66.
26. Maranhao de Moura AA, de Paiva JG. Pulpal calcifications in patients with coronary atherosclerosis. Endod Dent Traumatol 1987;3(6):307-9.
27. Howe P. The conservation of the dental pulp. Dent Items Interest 1919;41:933-5.
28. Bernick S. Age changes in the blood supply to human teeth. J Dent Res 1967;46(3):544-50.
29. Gold SI. Root canal calcification associated with prednisone therapy: A case report. J Am Dent Assoc 1989;119(4):523-5.
30. Nasstrom K, Forsberg B, Petersson A, Westesson PL. Narrowing of the dental pulp chamber in patients with renal diseases. Oral Surg Oral Med Oral Pathol 1985;59(3):242-6.
31. Galili D, Berger E, Kaufman E. Pulp narrowing in renal end stage and transplanted patients. J Endod 1991;17(9):442-3.
32. Ganibegovic M. Dental radiographic changes in chronic renal disease. Med Arh 2000;54(2):115-8.
33. Antonelli JR, Hottel TL. Oral manifestations of renal osteodystrophy: case report and review of the literature. Spec Care Dentist 2003;23(1):28-34.
34. Spolnik KJ, Patterson SS, Maxwell DR, Kleit SA, Cockerill EM. Dental radiographic manifestations of end-stage renal disease. Dent Radiogr Photogr 1981;54(2):21-31.
35. Nordstrom BL, Kinnunen T, Utman CH, Krall EA, Vokonas PS, Garvey AJ. Predictors of continued smoking over 25 years of follow-up in the normative aging study. Am J Pub Heal 2000;90(3):404-6.
36. Lawrence HP, Garcia RI, Essick GK, Hawkins R, Krall EA, Spiro A, 3rd, et al. A longitudinal study of the association between tooth loss and age-related hearing loss. Spec Care Dent 2001;21(4):129-40.
37. Krall EA, Garvey AJ, Garcia RI. Smoking relapse after 2 years of abstinence: Findings from the VA Normative Aging Study. Nicotine Tob Res 2002;4(1):95-100.

38. Garcia RI, Krall EA, Vokonas PS. Periodontal disease and mortality from all causes in the VA Dental Longitudinal Study. Ann Periodontol 1998;3(1):339-49.
39. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). Jama 2001;285(19):2486-97.
40. Carey V, Zeger, S.L., and Diggle, P. Modelling multivariate binary data with alternating logistic regressions. Biometrika 1993;80:517 -26.
41. Khosrovani A. A Retrospective Longitudinal Study of the Possible Effect of a History of a Myocardial Infart on Root Canal Occlusion (Calcification) in Older Men. [San Francisco: University of California San Francisco; 2003.
42. Woo S. Secondary Dentin Formation and Root Canal Occlusion in Middle-Aged to Elderly Men in a VA Dental Longitudinal Study [San Francisco: UC San Francisco; 2001.
43. Kaffe I. Endodontic Radiology. In: Goaz P WS, editor. Oral Radiology Principles and Interpretation. 2nd ed. St. Louis: C.V. Mosby; 1987. p. 268-98.