Original Article

Determination of efficacy of root planing in removal of nicotine from periodontally involved teeth of smokers

Abstract

Background and Aims: Tobacco smoking is now recognized to be an important risk factor for the development and progression of periodontal disease. Nicotine, the major constituent of particulate phase of tobacco smoke, in addition to having its toxic systemic effects, is capable of causing local cytotoxicity. The typical characteristic of smoking-associated periodontal disease is the destruction of the supporting tissues of the teeth, with the ensuing clinical symptoms of bone loss, attachment loss, pocket formation, and eventually tooth loss. The mechanisms behind the destructive effects of smoking on the periodontal tissues, however, are not well understood. This study aimed to detect nicotine from the root surfaces of periodontally involved root surfaces and to compare the quantity of nicotine present on root-planed and non-root-planed surfaces of teeth from smokers. **Materials and Methods:** 25 periodontally involved extracted teeth were taken from 18 smoker patients. The roots were sectioned longitudinally and each root half was either root planed (group B) or left untreated (group A). Each root half was extracted for nicotine using methylene chloride technique, and quantified using high pressure liquid chromatography (HPLC). **Statistical analysis:** Nicotine concentrations were compared between the root planed ans the non root planed groups using paired t-test. **Results:** The results showed that nicotine could be detected from the root surface of periodontally involved teeth. The amount of nicotine present on non-root planed sections was statistically significantly higher than on treated sections. **Conclusion:** Nicotine is present on the periodonatally involved root surfaces of smoker patients and also its concentration can be significantly reduced by thorough root planning.

Key words:
High-pressure liquid chromatography, nicotine, periodontal disease, root planing, tobacco smoking

Introduction

Cigarette smoking is the single most important and modifiable factor responsible for lung cancer, hypertension, and cardiovascular disease.[1] Malignant and premalignant lesions have been associated with cigarette smoking. Periodontal disease has been added to the ever-increasing list of health consequences (oral and systemic) of tobacco smoking. Chronic exposure to tobacco and its byproducts significantly affect the prevalence and progression of periodontal diseases.[2] In addition, tobacco use complicates periodontal therapy[3] and substantially reduces the possibility of favorable treatment outcomes.[4]

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Among all these substances found in tobacco smoke is the alkaloid "nicotine," which appears to be responsible for the dependence that characterizes the smoking habit.[5,6] The oral tissues of smokers are exposed to very high nicotine concentrations that negatively affect the cell population. Gingival crevicular fluid nicotine concentrations can be up to nearly 300 times that of nicotine plasma concentrations in smokers (20 ng/ml).[7] The vasoconstrictive properties of nicotine are hypothesized to impair gingival blood flow. Smoking has been shown to impair revascularization during soft and hard tissue wound healing which is critical for periodontal plastic surgery, regenerative, and implant

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procedures.[8] This might explain the diminished treatment response to surgical procedures, especially those involving tissue engineering.

The deleterious effects of smoking appear to result in part from down-regulation of the immune response to bacterial challenge. Neutrophils obtained from the peripheral blood, oral cavity, and saliva of smokers have been shown to demonstrate functional abnormalities in chemotaxis, phagocytosis, and oxidative burst.[9] Further, increased levels of tissue destructive enzymes such as tumor necrosis factor alpha (TNF-α),[10] prostaglandin E_2 (PGE_2), neutrophil elastase, and matrix metalloproteinase-8 have been demonstrated in the gingival crevicular fluid of smokers.[11]

Nicotine binds to the root surface of smokers[12] and *in vitro* studies have shown it can alter fibroblast attachment[13,14] and integrin expression[15] and decrease collagen production while increasing collagenase production. Root surfaces of teeth extracted from smokers show reduced periodontal ligament attachment as compared to those from non smokers.[16] Furthermore there is evidence of a synergistic effect on inflammatory mediator production when bacterial lipopolysaccharide is combined with nicotine.[17] Root surface of smokers that is affected by periodontitis contains toxic substances derived from two main sources: plaque and tobacco. Anaerobic plaque is a source of potent cytotoxic substance that may be endotoxin or related substance.[18] Nicotine was found to accumulate in significant amounts on the periodontally involved roots of smokers. The presence of nicotine on root surfaces with its harmful effects on those factors intimately associated with periodontal health should also be of some concern. Indeed, it could be the presence of nicotine on root surfaces which makes the smoking patient more disease susceptible and delay healing following non-surgical and surgical therapy and might explain increased tissue destruction observed in smokers.

Scaling and root planing are the two most commonly used therapeutic methods to remove toxic substances from periodontitis affected root surfaces and make them biologically acceptable substrate for healing of periodontal tissues. Hence, this study aimed to detect the presence of nicotine and assess the efficacy of root planing in its removal from the periodontally involved teeth of smokers.

Materials and Methods

This study was conducted at the Department of Periodontics, K.L.E.S's Institute of Dental Sciences, Belgaum, Karnataka. Twenty-five single rooted teeth, extracted due to periodontal reasons, were analyzed for this study. The teeth were obtained from 18 smoker patients smoking more than 20 cigarettes/day for ≥2 years. Patients were excluded if they had undergone professional oral prophylaxis 6 months before the study. Each patient contributed one to three teeth [Table 1]. The roots were sectioned in half longitudinally. One half of each root was extensively root planed to remove the outer surface of the root. Teeth were instrumented until the surfaces were determined to be hard and smooth. This was performed with ultrasonic scalers using highest power setting, copious water flow (Cavitron Dentsply, New York, NY). The other half of the tooth was not manipulated, except for the removal of soft tissue and served as untreated control. The root halves were stored in saline at 0°C-5°C until further analysis.

Procedures

The chromatographic mobile phase, consisting of water/methanol/acetic acid (0.02 M)/acetonitrile, 59/29/102 (v/v/v/v), adjusted to pH 6.8 with triethylamine at room temperature, was degassed and filtered before use. The nicotine stock standard, 200 ng/ml in 20% (v/v) methanol/water, was kept refrigerated at 4°C until use.

Each root half was placed into a separate 15-ml centrifuge tube equipped with a Teflon-lined cap. 2 ml of 0.5 M HCl was added to each tube and the tubes were rotated for 4 hours. 1 ml of high-pressure liquid chromatography water as a blank and 1 ml of 200 ng/ml nicotine standard were also acidified with 2 ml of 0.5 M HCl and treated identically to the root halves. At the end of 4 hours, the mixtures were alkalinized with 1 ml 2.5 M KOH. Alkalinization

Table 1: Concentration of nicotine in non-root-planed root halves (Group A)

Patient	Serial No.	Result (ng)	Sample weight (g)	ng/g
A	1	41.08	0.212	193.77
B	2	123.35	0.258	478.1
	3	151.70	0.286	530.41
	4	97.16	0.221	439.63
C	5	47.93	0.258	185.78
D	6	80.66	0.289	279.09
E	7	125.64	0.245	512.81
F	8	0.00	0.212	0.00
G	9	43.09	0.254	169.65
	10	30.64	0.236	129.83
H	11	78.46	0.178	441.79
I	12	88.69	0.232	382.28
	13	99.00	0.254	389.63
J	14	0.00	0.192	0.00
	15	0.00	0.244	0.00
K	16	0.00	0.275	0.00
	17	0.00	0.192	0.00
L	18	79.74	0.231	345.19
M	19	98.27	0.234	419.97
N	20	153.86	0.277	555.44
O	21	0.00	0.243	0.00
	22	0.00	0.265	0.00
P	23	51.76	0.186	278.27
Q	24	71.97	0.253	284.27
R	25	77.45	0.212	365.34

resulted in a precipitation of calcium phosphate at the root-half tubes. 5 ml of methylene chloride was added to each tube, and the nicotine was extracted by pulse vortexing for 2 minutes. Centrifugation at 2000 rpm for 10 minutes effectively separated the phases and caused the calcium phosphate precipitate to deposit as a disk between the two phases. Longer centrifugation times or higher rpm tended to cause the precipitate to descend into the methylene chloride phase, making it more difficult to remove. The upper aqueous layer was removed by aspiration with a glass transfer pipette and discarded. The precipitate disk in each root-half tube was gently pushed aside with the pipette and the methylene chloride poured off into a 13 × 100 mm test tube. 4 ml of the organic phase was pipetted into a clean 10 ml conical centrifuge tube. 100 µl of 0.2 M methanolic HCl was added to each tube to convert nicotine to its salt form, and the methylene chloride was gently removed under nitrogen. The resulting extract was redissolved in 250 µ1 of mobile phase before chromatography.

Nicotine concentrations in the extracted samples were calculated from peak areas compared with the nicotine standard (100 ng/ml). The amount of nicotine in the reconstituted sample was calculated and divided by the weight of the root half to yield nicotine concentrations of ng nicotine/g root half. The nicotine standard curve was linear up to 200 ng/ml (r = 0.999). The limit of detection was 0.5 ng/ml of extracted sample.

Statistical analysis

Nicotine concentrations as ng extracted nicotine/g tooth were compared between the root-planed and the non-root-planed tooth root halves by a paired t-test and Wilcoxin sign test.

The level of significance of "P" value at 95% confidence interval was calibrated as follows:

Not significant (NS)	: $P>0.05$
Significant (S)	: $0.01 < P<0.05$
Very significant (VS)	: $0.001 < P<0.01$
Highly significant (HS)	: $P<0.001$

Results

The paired t-test showed a highly significant difference in nicotine levels between the two halves ($P<0.0005$). Additionally, the Wilcoxin sign test also showed a highly significant difference between root-planed and non-root-planed halves ($P<0.0001$).

The chromatographic tracing of the nicotine standard [Figure 1] shows distinct absorbance by nicotine at approximately 6 minutes elution time. The identity of the nicotine peak was confirmed by a Photodiode Array Detector, which yields a complete UV spectrum which was identical to reference library spectrum. [Figures 2 and 3] show the chromatographic tracing of extract of nicotine from untreated and treated root sections, respectively.

[Table 1] denotes the nicotine concentration of all the 25 samples before root planing (Group A). The highest and least levels of nicotine in the non-root planed (Group A) were 555.44 and 129.83 ng/g, respectively, with a mean of 255.25 (±195.9). The mean weight of the samples was 0.237 g (±0.031 g). The standard sample of nicotine used was of 52637 (mean of 51,674 and 53,600).

[Table 2] shows nicotine concentration of all the 25 samples after root planing (Group B). The highest and least levels of nicotine in the non root planed (Group B) were 483.73 and 0.00 ng/g, respectively, with a mean of 71.45 (±148.8). The

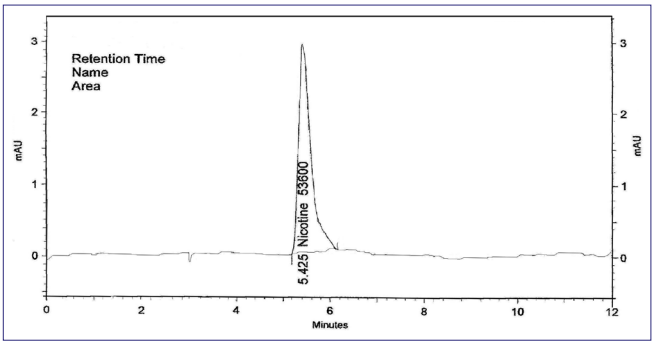

Figure 1: Chromatographic tracing of nicotine standard (200 ng)

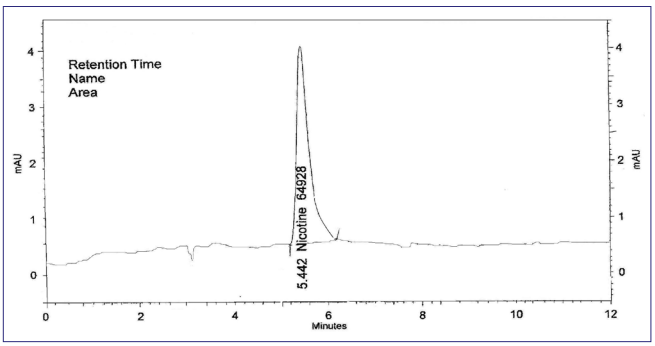

Figure 2: Chromatographic tracing of nicotine extract from untreated root section

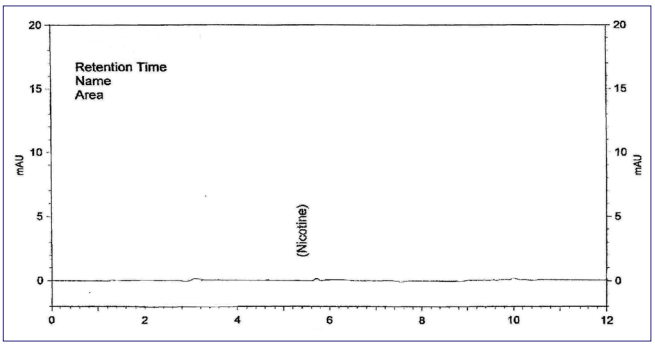

Figure 3: Chromatographic tracing of nicotine extract from treated root section

mean weight of the samples after root planning was 0.182 g (±0.019 g). The standard sample of nicotine used was of 32073 (mean of 32,750 and 31,396).

[Table 3] shows the difference in nicotine concentration before and after root planing. While nicotine was detected in 20 patients before root planing, it was seen in only five root halves after the treatment. These five treated halves still harbored nicotine on root surfaces, but their concentration was significantly decreased when compared with their untreated counterparts (*P*=0.0005).

Discussion

Tobacco smoking is now recognized to be an important risk factor for the development and progression of periodontal disease.[19] In addition, there is ample evidence to support the concept that smoking influences periodontal healing responses following periodontal therapy.[20,21] Numerous studies of potential mechanisms whereby smoking tobacco may predispose to periodontal disease have been conducted. Smoking exerts a deleterious effect on the protective elements of the immune response, resulting in an increase in the extent and severity of periodontal destruction. It impairs the response of neutrophils to periodontal infection[9] as well as increases the release of proinflammatory cytokines.[10] Tobacco smoke contains cytotoxic and vasoactive substances, including nicotine, which may mediate the local affects in the periodontium.

The results of this study have shown that nicotine is present on roots of periodontally diseased teeth in smokers and also that thorough root planing significantly reduces its concentration on the root surfaces.

In non-root-planed group (Group A), the mean concentration of nicotine was 255.25 ng/g. In this study, the mean concentration of nicotine in the Group A was 255.25 ng/g. This study is in accordance with the study done by Cuff *et al*.,[12] who also aimed to detect nicotine from the periodontally involved root surfaces of smokers. The authors reported a mean nicotine concentration of 144 ng/g in their study. This higher range of nicotine reported in this study could be attributed to the selection of smoker subjects, who were strictly heavy smokers, smoking ≥20 cigarettes/day. The periodontal destruction in smokers appears to be dose dependent, with heavy smokers more susceptible to the destructive effects of nicotine when compared with light and moderate smokers.[22] Thus, the criteria of heavy smokers were set with an objective of detecting nicotine concentrations on the root surfaces of heavy smokers and to what extent it could be reduced by thorough root planning in order to create an environment conducive for attachment of cells to occur during healing.

Out of the entire root-planed samples (Group B), all but

Table 2: Concentration of nicotine in root-planed root halves (group B)

Patient	Serial No.	Result (ng)	Sample weight (g)	ng/g
A	1	0.00	0.152	0.00
B	2	0.00	0.185	0.00
	3	88.37	0.242	308.97
	4	61.65	0.178	346.34
C	5	0.00	0.189	0.00
D	6	52.48	0.195	0.00
E	7	0.00	0.175	0.00
F	8	0.00	0.163	0.00
G	9	0.00	0.215	0.00
	10	0.00	0.172	0.00
H	11	52.15	0.164	317.98
I	12	60.92	0.185	329.29
	13	0.00	0.171	0.00
J	14	0.00	0.192	0.00
	15	0.00	0.185	0.00
K	16	0.00	0.194	0.00
	17	0.00	0.163	0.00
L	18	0.00	0.216	0.00
M	19	0.00	0.167	0.00
N	20	84.65	0.175	483.73
O	21	0.00	0.193	0.00
	22	0.00	0.175	0.00
P	23	0.00	0.163	0.00
Q	24	0.00	0.172	0.00
R	25	0.00	0.188	0.00

Table 3: Difference in nicotine concentration before and after root planing

Sample No.	Group A (ng/g)	Group B (ng/g)
1	193.77	0.00
2	478.1	0.00
3	530.41	308.97
4	439.63	346.34
5	185.78	0.00
6	279.09	0.00
7	512.81	0.00
8	0.00	0.00
9	169.65	0.00
10	129.83	0.00
11	441.79	317.98
12	382.28	329.29
13	389.63	0.00
14	0.00	0.00
15	0.00	0.00
16	0.00	0.00
17	0.00	0.00
18	345.19	0.00
19	419.97	0.00
20	555.44	483.73
21	0.00	0.00
22	0.00	0.00
23	278.27	0.00
24	284.27	0.00
25	365.34	0.00

five of them showed the effect of thorough root planing in significantly reducing the amount of nicotine adhering to the root surface. A significant reduction of nicotine after thorough root planing suggests that nicotine, like endotoxin,[23,24] is adsorbed or is loosely adherent to the root surface. That some nicotine was still detectable on five of the samples after treatment suggests either deep penetration of the substance into the root or diffusion outward from the pulp. Regardless of the mechanism of deposition, the concentration at the surface was generally diminished in all the root samples following root planning. Similar results were reported by Cuff *et al*.,[12] who reported a statistically significant reduction in nicotine concentration after thorough root planning.

In this study, the highest and lowest concentrations of nicotine reported were 555.45 and 129.8 ng/g in the untreated group (Group A) and the mean being 255.25 ng/g (±195.9). Since nicotine has a short half-life, it would appear that relating the time of patient's last cigarette to the time of tooth extraction could account for the variable levels of nicotine detected in the samples. Considering this, there are several other variables, which could be considered. This includes the brands of cigarette smoked, the presence and types of filter, and the accuracy of self-reporting.[12] Since adjusting all these variables was not practical, no attempt was made to correlate nicotine concentration between teeth or smokers, but comparison was made just between the treated and untreated samples of individual patient.

Smokers compared to age-matched groups of non-smokers exhibit greater prevalence, extent, and severity of recession.[25-27] The strong association between smoking and gingival recession appears independent of interproximal attachment loss and dependent on overall severity of tobacco exposure.[13] Nicotine inhibits the proliferation, adhesion and chemotaxis of periodontal ligament cells, alters the interaction between epithelial cells and gingival fibroblasts,[28] increases gingival fibroblast collagenolytic activity,[6] and inhibits the adhesion of fibroblasts to root surfaces.[16] These changes could all contribute to more destruction seen in smokers.

Smoking negatively impacts the gingival blood flow, a factor critical for proper periodontal flap healing.[6] Numerous studies have shown that smoking compromises probing depth and/or attachment gain outcome following surgical and non-surgical therapy. Smokers, especially heavy smokers, represent a population with greater root coverage treatment needs.[6] The potential negative impact of smoking on the outcome of root coverage procedures was recognized by Miller almost 20 years ago.[29] Collectively, these studies show that probing depth reduction and clinical attachment level improvements are 50%–75% those of non-smokers following non-surgical and surgical periodontal therapy. In terms of dose response, a trend, albeit not significant at most points, has been noted for heavy smokers to respond less favorably than light smokers.

The main objective of root planing has been to remove plaque, calculus, toxic agents, and altered cementum from pathologically exposed root surface, thereby rendering it "biologically acceptable" for optimal wound healing. Nicotine on root surfaces of periodontally involved teeth and its effects on factors intimately associated with periodontal health and optimal wound healing should also be of concern. The reason for the smokers to respond less favorably than non-smokers to the various periodontal treatment modalities may partially be due to the presence of nicotine in the wound-healing arena. The challenge to the clinician then is to eliminate nicotine levels on the root surface throughout the period of wound healing following periodontal therapy. The obvious solution is to have the patient abstain from smoking during active periodontal therapy to include the immediate postoperative period. The current finding that concentration of nicotine can be significantly diminished by thorough root planing substantiates this mode of therapy.

The findings of this study justify the clinical benefits obtained by scaling and root planing in smokers. Although smokers showed a consistent decreased gain in clinical attachment levels and probing depths when compared with non-smokers,[8] periodontal therapy showed some positive response in treated smokers when compared with non-treated smokers. When a higher level of plaque control can be achieved as a part of non-surgical care, the difference in resolution of 4–6 mm pockets between smokers and non-smokers become clinically less significant.[29] Smokers respond less well to non-surgical therapy than non-smokers; however, these differences may be minimized with excellent plaque control.

Cotinine, one of the metabolites of nicotine, in body fluids is considered an accurate measurement of smoking or exposure to smoke.[30] Self-reported cigarette consumption may be an inaccurate measurement of how much an individual smokes. This may result in erroneous calculation of nicotine exposure. Hence, further studies should be conducted by using cotinine as a chemical marker to standardize nicotine exposure rather than the self-reported histories. In this way, the errors arising due to the brand of cigarette smoked and the type of filters present in them can also be avoided.

Conclusion

Thus, we conclude that nicotine could be detected from the root surfaces of periodontally involved teeth of smokers and its concentration can be greatly reduced by thorough root planing. Considering the number of potential deleterious effects of nicotine on periodontium, root surface nicotine could well be one of the pathways by which tobacco smoke

exerts its local deleterious effects and its elimination from root surfaces should be the goal of the clinician throughout the period of active periodontal therapy and immediate postoperative period during wound healing.

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