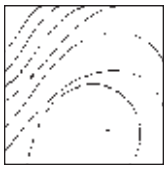
Impact of Interleukin 1 Gene Polymorphism and Smoking on Long-Term Stability Following Gingival Recession Treatment



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Risk factors such as smoking, genetic factors, and tissue biotype play an important role in the etiology, predictability, and long-term stability of gingival recession treatment. This study was designed to evaluate the influence of interleukin 1 (IL-1) polymorphism and smoking on the stability of gingival recession treatment after 1 and 3 years. All patients (n = 55) were treated for type I and II recession defects using a connective tissue graft. Clinical evaluations were performed, which included assessment of vertical recession depth, gingival inflammation, and clinical attachment level. A fingerstick blood sample was collected using specially provided DNA filter paper and mailed for processing in a laboratory using polymerase chain reaction–based methodology. The results indicated that 19 subjects were genotype positive (34.5%). Treatment of the localized recessions was effective and provided a similar amount of coverage in genotype-positive and genotype-negative subjects within smoking and nonsmoking groups after 1 year. In a 3-year period, nonsmoking patients with positive IL-1 genotype lost approximately 20% of the root coverage gained at 1 year and were almost four times more inferior compared with genotype-negative patients. Patients who smoked and had a positive IL-1 genotype lost approximately 35% of the gained root coverage. IL-1 polymorphism and smoking habit did not affect gingival recession treatment at 1 year but had a great impact on long-term stability.
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The occurrence rate of gingival recession for adults over the age of 30 years is nearly 60%.[1] Common risk factors such as smoking, genetic factors, and tissue biotype play an important role in the etiology, predictability of therapy results, and long-term stability of gingival recession treatment. The impact of interleukin 1 (IL-1) polymorphism holds a special place as a risk factor related to periodontal therapy. The predominant form of IL-1 in the periodontal tissues is IL-1β. Because of its multiple proinflammatory properties, it has a key role in the pathogenesis of periodontitis. Genotype-positive patients produce increased levels of proinflamatory IL-1 in response to inflammatory stimuli, which can lead to greater destruction of periodontal tissues and compromised healing and tissue regeneration.[2–6]

Kornman et al[7] evaluated the presence of a genetic marker for the severity of periodontal disease and found a significant association between severe periodontitis and the composite genotype that comprises allele 2 of the –889 IL-1α polymorphism as well as allele 2 of the +3954 IL-1β polymorphism (IL-1α2/IL-1β2).

Several reports have assessed the prevalence of this IL-1 periodontal genotype.[2,8–11] Kornman et al[7] also reported that 29.1% of the Northern European Caucasian population was evaluated as being genotype positive (at least one allele 2 present at each locus). Lang et al[2] reported 35.3% of a Swiss maintenance population to be genotype positive. In the United States, between 29% and 38% of subjects have been reported to be genotype positive.[9–11] Caffesse et al[12] found a 26% prevalence of genotype-positive subjects in a Mexican population. In African-American and Chinese populations, the prevalence of genotype-positive individuals has been reported to be reduced.[8,13] Genotype-positive subjects have been described as presenting more rapid periodontal breakdown, increased bleeding on probing, and a four-times increased production of IL-1 by monocytes to the same bacterial challenge.[2,7,14] Few studies have reported the response to therapy in subjects who are genetically positive.[5,15–18] Some studies have found that IL-1α and IL-1β polymorphisms are associated with a higher severity of periodontal diseases,[3,19,20] while others presented no association between these polymorphisms and incidence, onset, or severity of disease.[6,21,22] De Sanctis and Zucchelli[5] reported that genotype expression did not affect the response to guided tissue regeneration therapy at 1 year but had a great impact on long-term stability (4 years).

To the authors' knowledge, only one study has been published evaluating the association between IL-1 polymorphism and gingival recession treatment results. Caffese et al[18] presented results of gingival recession treatment in a periodontally healthy Hispanic population with IL-1 gene polymorphism.

Smoking is known to be an important risk factor for periodontal breakdown.[23,24] Experimental evidence has demonstrated that cigarette smoking may negatively influence healing outcomes following various periodontal therapeutic procedures. Root coverage with connective tissue grafts appears to be negatively associated with cigarette smoking.[25,26] It has been shown that heavy smoking neutralizes the effects of a genotype-negative (disease-resistant) patient. Positive IL-1 genotype in combination with smoking has influenced tooth loss after periodontal therapy both independently and combined (7.7-times greater likelihood).[7]

The purpose of this study was to evaluate the impact of IL-1 polymorphism and smoking on the results and long-term stability (3 years) of gingival recession treatment in periodontaly healthy patients.

Method and materials

Fifty-five patients (29 men, 26 women; mean age, 30.89 years; range, 20 to 41 years) from the Clinic for Periodontology, School of Dentistry, University of Belgrade, Belgrade, Serbia, were consecutively enrolled in this clinical study. The university ethical committee approved the protocol for human subjects. Prior to their selection, all patients completed a routine questionnaire, from which a general medical history and patient demographics including age and sex were obtained. Thirty subjects were nonsmokers and 25 were smokers. Patients were regarded as smokers if they smoked at least 20 cigarettes per day. The nonsmokers did not smoke at all. All patients were systemically healthy and without any significant history of systemic disease. Patients diagnosed with periodontitis were excluded. Study participants were all treated more than 3 years prior with subepithelial connective tissue grafts in combination with coronally advanced flaps[27] for the coverage of a type I or II localized gingival recession.[28] All surgical procedures were performed by the same operator, who was not involved with clinical measurements.

All patients underwent full-mouth dental and periodontal examinations, which included assessment of gingival inflammation using the Gingival Index (GI)[29] and clinical attachment levels (CAL). Status of gingival recessions was evaluated using the vertical gingival recession depth (VRD), measured as the distance from the cementoenamel junction to the free gingival margin (the middle point of the exposed root was considered). Recordings were performed at baseline and 1 and 3 years after mucogingival surgery. All clinical data were collected pre- and postsurgically by the same periodontist. who was unaware of the patients' smoking and IL-1 polymorphism history and who was not involved with the surgery. After

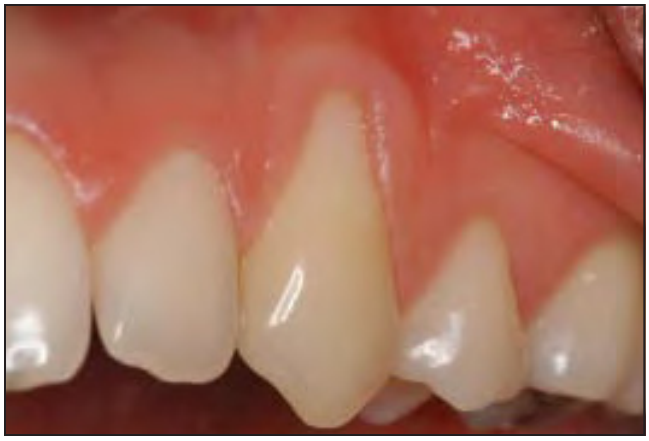

Fig 1a *Preoperative view showing gingival recession at a maxillary left canine (nonsmoker, IL-1 genotype negative).*

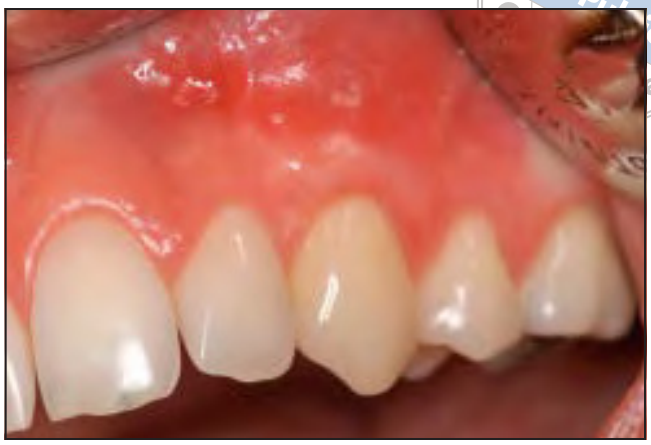

Fig 1b *Three-year postoperative view showing complete root coverage.*

surgical treatment, patients were placed on regular preventive maintenance schedules with prophylaxis every 3 to 6 months.

Participants were divided into smoking and nonsmoking groups. At the 3-year examination, all patients were tested for the IL-1 genotype using a fingerstick blood draw following the manufacturer's indications. The tip of the middle finger was cleaned with an antiseptic wipe. A fingerstick was produced with a lancet, and the finger was squeezed to promote bleeding. The blood was collected on DNA-ase-free blotting paper and sent for blinded analysis to the genetic testing laboratory. The results were reported as positive or negative for the IL-1 gene polymorphism depending on the presence of allele 2 in both IL-1α and IL-1β genes. The blood sample was allowed to dry for several hours, after which the collection card was closed and the code identified. The card was then mailed for processing in a laboratory using polymerase chain reaction–based methodology.

The gain in recession coverage was evaluated for both genotype-positive and genotype-negative subjects in the smoking and nonsmoking groups, and the mean percentages of coverage were statistically analyzed using the Student *t* test.

Results

The results indicated that 19 of 55 subjects (34.5%) were genotype positive. Twenty-five subjects (45.45%) were smokers.

Mean VRD results in the nonsmoking group at baseline showed no statistically significant differences between genotype-positive (3.10 ± 0.87 mm) and genotype-negative patients (3.35 ± 0.67 mm) (Figs 1a and 2a). After 1 year, the mean VRD in genotype-positive subjects was 0.30 ± 0.48 mm, or 92% root coverage. At the same time, the mean VRD in genotype-negative subjects was 0.25 ± 0.44 mm, or 93.2% root coverage. No statistically significant difference in percent root coverage between genotype-positive and genotype-negative subjects was detected ($P = .4209$). The mean change in VRD for genotype-positive subjects was 2.80 ± 0.63 mm, while that for genotype-negative subjects was 3.10 ± 0.64 mm. Three years after surgery, the mean VRD in genotype-positive subjects was 0.90 ± 0.73 mm, or 75% root coverage, while that obtained in genotype-negative subjects was 0.45 ± 0.51 mm, or 88% root coverage (Figs 1b and 2b). A statistically significant difference for percent root coverage between genotype-positive and genotype-negative subjects was detected ($P = .0317$). The mean reduction of VRD in genotype-positive and genotype-negative subjects after 3 years was 2.20 ± 0.42 and 2.95 ± 0.60 mm, respectively. Comparing the mean change in VRD for the genotype-positive group at 1 and 3 years after surgery, a statistically significant difference was recorded ($P = .011$); mean change in the genotype-negative group did not reach statistical significance ($P = .225$) (Table 1). Frequency dis-

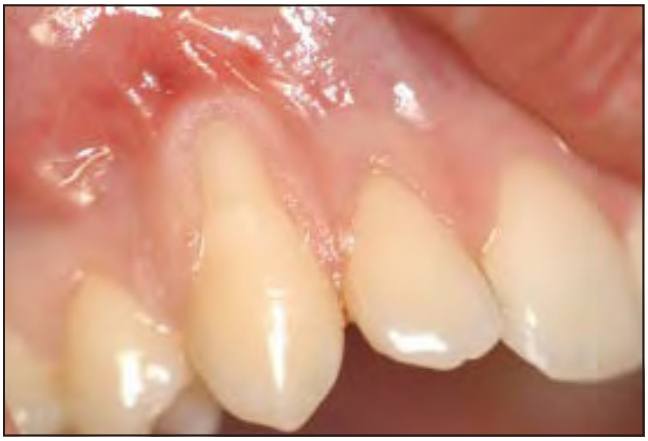

Fig 2a *Preoperative view showing gingival recession at a maxillary right canine (nonsmoker, IL-1 genotype positive).*

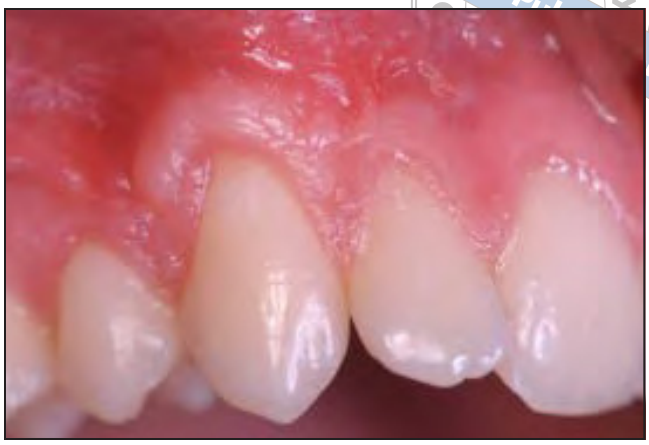

Fig 2b *Three-year postoperative view.*

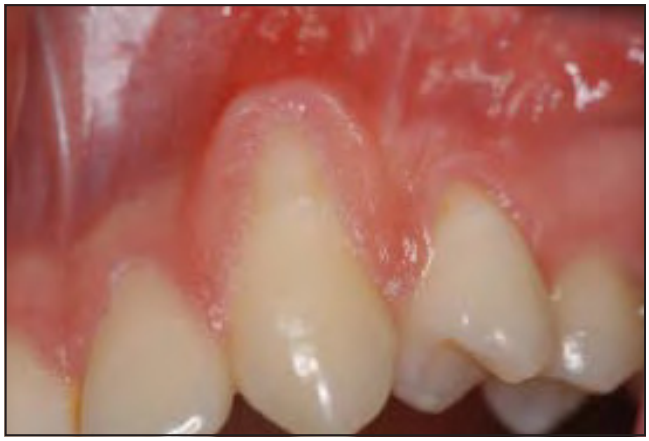

Fig 3a *Preoperative view showing gingival recession at a maxillary left canine (smoker, IL-1 genotype positive).*

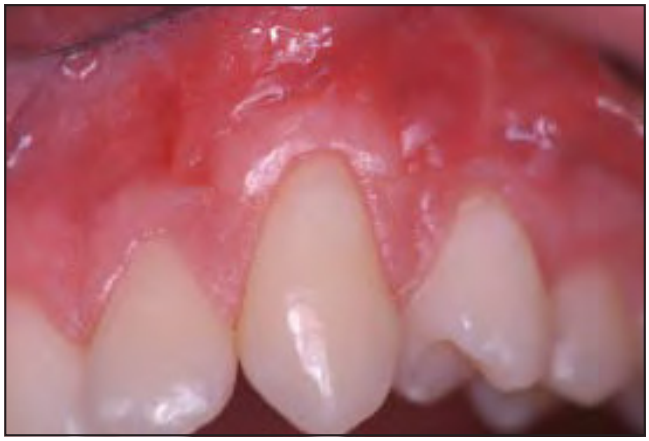

Fig 3b *Three-year postoperative view.*

tribution of complete root coverage according the genotype 1 year after the surgical procedure was 75% in genotype-negative and 70% in genotype-positive subjects. After 3 years, these values decreased to 55% and 30%, respectively.

Mean VRD results in the smoking group at baseline showed no statistically significant differences between genotype-positive (3.44 ± 0.53 mm) and genotype-negative patients (3.56 ± 0.63 mm). One year after surgery, no statistically significant difference in percent root coverage between genotype-positive (86%) and genotype-negative subjects (92%) was recorded (P = .201). Three years after the surgical procedure, the mean VRD in genotype-positive subjects was 1.55 ± 0.73 mm, or 57% root coverage, while that obtained in the genotype-negative group was 0.81 ± 0.54 mm, or 79% root coverage. A statistically significant difference for percent root coverage between genotype-positive and genotype-negative subjects was detected (P = .0008) (Table 2). Frequency distribution for complete root coverage according to genotype 1 year after the surgical procedure was 68.75% in genotype-negative and 55.55% in genotype-positive subjects. After 3 years, these values decreased to 25% and 0%, respectively.

Comparison of mean percent coverage obtained in the smoking and nonsmoking groups within genotype-negative and genotype-positive subjects showed no significant difference 1 year after surgery (genotype-negative, P = .459;

Table 1 Mean VRD for genotype-positive and genotype-negative nonsmokers

		1 y			3 y		
Genotype	Initial VRD	VRD	Coverage	% coverage	VRD	Coverage	% coverage
Negative	3.35 ± 0.67	0.25 ± 0.44	3.10 ± 0.64	93.2	0.45 ± 0.51	2.95 ± 0.60	88.0
Positive	3.10 ± 0.87	0.30 ± 0.48	2.80 ± 0.63	92.0	0.90 ± 0.74	2.20 ± 0.42	75.0
P	.220	.393	.119	.4209	.043*	.0002*	.03176*

VRD = vertical gingival recession depth.
*Statistically significant, $P < .05$.

Table 2 Mean VRD for genotype-positive and genotype-negative smokers

		1 y			3 y		
Genotype	Initial VRD	VRD	Coverage	% coverage	VRD	Coverage	% coverage
Negative	3.56 ± 0.63	0.31 ± 0.48	3.25 ± 0.57	92	0.81 ± 0.54	2.81 ± 0.40	79
Positive	3.44 ± 0.53	0.55 ± 0.72	2.89 ± 0.33	86	1.55 ± 0.73	1.88 ± 0.33	57
P	.311	.193	.059	.201	.009*	2.854E–06*	.0008*

VRD = vertical gingival recession depth.
*Statistically significant, $P < .05$.

Table 3 Comparison of root coverage between smokers and nonsmokers in genotype-negative and genotype-positive patients

			Root coverage (%)			
	Initial VRD (mm)		1 y		3 y	
	Negative	Positive	Negative	Positive	Negative	Positive
Nonsmokers	3.35 ± 0.67	3.10 ± 0.87	93.2	92.0	88.0	75.0
Smokers	3.56 ± 0.63	3.44 ± 0.53	92.0	86.0	79.0	57.0
P	.167	.155	.45961	.209155	.029805*	.01528*

VRD = vertical gingival recession depth.
*Statistically significant, $P < .05$.

genotype-positive, $P = .209$). Comparing data 3 years after root coverage procedures, a statistically significant difference was recorded within both genotype-negative ($P = .0298$) and genotype-positive subjects ($P = .0152$), with more favorable results obtained in the nonsmoking group (Table 3).

At baseline, the genotype-positive nonsmoking subjects presented the following mean values: GI = 0.80 ± 0.42 mm and CAL = 4.20 ± 0.78 mm. The values for the genotype-negative nonsmoking subjects before treatment were 0.55 ± 0.51 mm for GI and 4.55 ± 0.68 mm for CAL. No statistically significant differences were found when both groups were compared ($P > .05$). The same relationships were recorded in the smoking group. After observation periods of 1 and 3 years, no statistically significant differences for GI values were noticed when both groups (genotype-positive and genotype-

negative smokers and nonsmokers) were compared ($P > .05$).

Evaluation of mean change in CAL values 1 year after surgery showed no statistically significant difference between genotype-positive and genotype-negative nonsmoking (2.80 ± 0.63 and 3.20 ± 0.62 mm, respectively) and smoking subjects (2.91 ± 0.66 and 3.95 ± 0.62 mm, respectively). When comparing CAL results from genotype-positive and genotype-negative nonsmoking subjects 3 years after mucogingival surgery, a statistically significant difference was recorded favoring the genotype-negative group ($P < .05$). The same relationships were recorded in the smoking group. Genotype-positive nonsmoking subjects showed a significantly greater increase in CAL (0.90 ± 0.63 mm) than the genotype-negative group (0.15 ± 0.37 mm) ($P = .0055$). Genotype-positive smokers also presented a significantly greater increase in CAL (0.95 ± 0.75 mm) compared with the genotype-negative group (0.36 ± 0.42 mm) ($P = .0045$).

Discussion

A significant reduction in VRD and gain in CAL was evident at the 1-year follow-up for all patients, indicating that both genotype-negative and genotype-positive patients can be successfully treated with a connective tissue graft regardless of their smoking habits. In fact, at 1 year, the amount of CAL gain and VRD reduction did not differ statistically between genotype-positive and genotype-negative patients. On the basis of the results, it can be noted that nominally but not statistically better outcomes were achieved in genotype-negative smokers compared with genotype-positive smokers 1 year after surgery. In genotype-negative patients, smoking did not have a noteworthy impact on root coverage. Genotype-positive (disease-susceptible) and genotype-negative (disease-resistant) smokers and nonsmokers showed similar values for gingival inflammation. The results suggest that if adequate maintenance is provided, genotype and smoking habit do not seem to have any harmful effects on the periodontium 1 year after mucogingival treatment. These results are in complete agreement with data published by Caffesse et al[18] and present a concept for the influence genotyping has on chronic periodontal disease in adult subjects.

Three years after the surgical procedure, genotype-positive nonsmokers showed a significant increase in VRD compared with results achieved 1 year after surgery ($P = .0226$). At the same time, genotype-negative nonsmokers showed a significantly greater level of stability, with a mean increase in VRD of 0.25 mm. The amount of coverage obtained in genotype-negative (88%) and genotype-positive (75%) subjects reached statistical significance ($P = .0317$). Caffesse et al[18] presented coverage results comparing genotype-positive and genotype-negative subjects with no statistical significance. At the same time, genotype-positive and genotype-negative smokers showed a reduction in the mean root coverage achieved 3 years after the surgical procedure. It has to be emphasized that genotype-positive smokers clearly demonstrated significantly greater loss of root coverage (Figs 3a and 3b) compared with genotype-negative smokers. Complete coverage (100%) for genotype-negative subjects was achieved in 55% of nonsmokers and 25% of smokers. Complete root coverage for genotype-positive subjects was obtained in 30% of nonsmokers, and no subject had complete root coverage in the smoking group 3 years after surgery.

The results of this study demonstrate that genotype expression and smoking habit did not strongly affect the response to gingival recession treatment at 1 year but had a great impact on long-term stability (3 years), especially in smokers. In a 3-year period, nonsmoking patients with positive IL-1 genotype lost approximately 20% of the root coverage gained over the first year and were about four times more inferior compared to genotype-negative patients. At the same time, smokers with a positive IL-1 genotype lost approximately 35% of the root coverage gained.

In this study, smokers who were negative and positive for the IL-1 genotype presented with a significantly increased risk for root coverage failure compared with nonsmoking patients 3 years after surgical treatment. When the IL-1 genotype was excluded from evaluation, smokers showed a signifi-

cantly lower level of root coverage stability (71%) than the nonsmoking group after 3 years (83%). Martins et al[26] presented a mean root coverage of 58.84% in patients who smoked 120 days after surgery.

The impact of IL-1 gene polymorphism and smoking habit on the results of gingival recession treatment was not evaluated for light smokers (fewer than 10 cigarettes per day). For a more precise assessment of the influence of IL-1 gene polymorphism and smoking on the stability of the results following gingival recession treatment, future studies should include and evaluate this smoking population.

Assessment of gingival tissue thickness was not performed in this study, but upcoming studies should evaluate this parameter as a promising potential predictor[30,31] of root coverage stability.

The results sustain the contention that genotype-positive patients with smoking habits are more susceptible to periodontal breakdown after root coverage procedures than genotype-negative nonsmoking subjects and emphasize the need for more aggressive supportive periodontal therapy in maintaining these patients. Gingival recession treatment procedures using connective tissue grafts are most likely to need additional grafting at some time in the future for IL-1–positive smokers.

Acknowledgment

The authors reported no conflicts of interest related to this study.

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