

ABSTRACT

The cytokines IL-1 β and IL-6 play significant roles in the pathophysiology of periodontitis. A C→T single nucleotide polymorphism (SNP) at position +3953 of the IL-1 β gene has been associated in several studies with increased periodontitis severity. A C→G SNP at position -174 within the IL-6 promoter is associated with increased transcriptional activity of the IL-6 gene. **Objectives:** This cross-sectional survey study was designed to determine if these two SNPs might influence the relationship between measured whole-mouth average Löe-Silness gingival index (GI) and pocket depth (PD). **Methods:** Subjects (N=340, mean age 45±12 yrs; 72% female; 60% nonsmokers) were enrolled in the study and genotypes determined using restriction fragment length polymorphism of gene-specific PCR products. Subjects (N=40) homozygote for allele 1 (IL-1 β CC, IL-6 CC) of both genes were classified as having no polymorphism (NP). Subjects (N=300) having at least one copy of allele 2 of either or both genes were classified as having a polymorphism (WP). The relationship between PD and GI was modeled with multiple regression analysis. **Results:** There was a significant positive correlation between PD and GI ($r=0.40$, $p<0.001$) in the WP group, but not in the NP group ($r=0.05$, $p=0.76$). Additionally, the slope of the linear relationship between PD and GI was significantly ($p=0.009$) greater in the WP group (slope=0.30mm, $p<0.001$) than in the NP group (slope=0.03mm, $p=0.8$). Results were similar in statistical models that adjusted for age and smoking history. Significantly ($p=0.003$) more females (91%) were WP vs. males (80%). **Conclusions: It appears that the association between PD and GI is greater for individuals with the IL-1 β /IL-6 WP composite genotype than for those without the composite genotype.**

INTRODUCTION

Gingivitis and periodontitis are infectious inflammatory diseases of the periodontium. Specific periodontal pathogens in dental plaque initiate an inflammatory response in the gingiva that can lead to the development of gingivitis. However, some patients develop periodontitis which is characterized by bone and connective tissue loss surrounding the teeth.

Environmental factors (e.g, oral hygiene, smoking) and health status (e.g, osteoporosis, diabetes) contribute to the risk of developing severe disease. Genetic modifiers are known to make an important contribution in determining the host response to bacterial challenge. Therefore, specific genetic markers may identify patients at elevated risk for severe disease. The cytokines IL-1 β and IL-6 play significant roles in the pathophysiology of periodontitis.

Recently, a C→T single nucleotide polymorphism (SNP) at position +3953 of the IL-1 β gene has been associated in several studies with increased periodontitis severity¹. A C→G SNP at position -174 within the IL-6 promoter is associated with increased transcriptional activity of the IL-6 gene². The study reported here was part of a larger cross-sectional survey study and was designed to determine if SNPs of various genes such as IL-1 β and IL-6 might influence the relationship between clinical measures of inflammation and periodontal tissue destruction such as gingival index (GI) and mean pocket depth (PD).

MATERIALS AND METHODS

The protocol for the cross-sectional survey study of which these analyses were a part was reviewed and approved by an Investigational Review Board (IRB). Informed consent was obtained by all study participants prior to participation in the study.

A total of 340 subjects were evaluated in this study (mean age 45±12 yrs; 72% female; 60% nonsmokers). These subjects were part of a larger cross-sectional survey study examining the relationship between periodontal health, overall health status, and habits and practices.

Venous blood samples were spotted on coded Isocode Stix™ blood collection cards. Sample coding was used to maintain sample blinding during genotype analysis. DNA was extracted from the card as per the manufacturer's instructions.

DNA was analyzed for SNPs using restriction fragment length polymorphism of gene-specific PCR products as previously described^{1,2}. Restriction fragments were resolved and identified using 20% TBE PAGE. For analysis, subjects having at least one copy of allele 2 from both IL-1 β and IL-6 genes were classified as WP and the others as NP.

IL-1 β +3953 C→T SNP

Digestion of the PCR product with TAQ1

- Allele 1 has a 'C' in the SNP position yielding 12, 85, and 97 BP fragments
- Allele 2 has a 'T' in the SNP position yielding 12 and 182 BP fragments

IL-6 -174 C→G SNP

Digestion of the PCR product with SfaN1

- Allele 1 has a 'C' in the SNP position yielding an uncut 198 BP fragment
- Allele 2 has a 'G' in the SNP position yielding 58 and 140 BP fragments

Gingival Index and Pocket Depth Measurements GI and PD were scored on or adjacent to six surfaces (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) of all natural teeth, excluding third molars, in the upper and lower jaw by a calibrated examiner. GI was measured according to the method of Löe-Silness (table 1). Single pass, whole mouth measures of pocket depth were made using a manual, incremental UNC-15 periodontal probe. The distance from the gingival margin to the base of the sulcus was defined as the PD. Average GI and PD scores and measures were derived for each subject by summing the individual scores and measures and dividing that sum by the number of sites graded for that subject.

MATERIALS AND METHODS (cont.)

Table 1: Loe-Silness Scoring Criteria

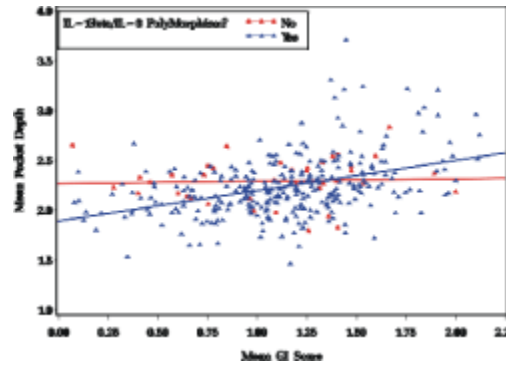
Score	Criteria
0	Absence of inflammation.
1	Mild inflammation: Moderate glazing, slight change in color, slight edema. No bleeding on probing.
2	Mild inflammation: Moderate glazing, redness, edema, and hypertrophy. Bleeding on probing.
3	Severe inflammation: There is marked redness, hypertrophy, and ulceration. Tendency to spontaneous bleeding.

RESULTS

Association between PD and GI in subjects with and without polymorphisms:

There was a significant positive correlation between PD and GI ($r=0.40$, $p<0.001$) in the WP group, but not in the NP group ($r=0.05$, $p=0.76$). The slope of the linear relationship between PD and GI was significantly greater ($p=0.009$) in the WP group than in the NP group. Results were similar in models that adjusted for age and smoking history. Significantly ($p=0.003$) more females (91%) were WP vs. males (80%).

Group	WP (Y/N)	N	Slope (PD vs. GI)	p-Value
All Subjects	No	40	0.025	0.8
All Subjects	Yes	300	0.30	<0.001
Non-Smokers	No	25	-0.03	0.77
Non-Smokers	Yes	178	0.20	0.001
Smokers	No	15	0.19	0.31
Smokers	Yes	122	0.36	<0.001



CONCLUSION

The association between PD and GI is significantly greater for individuals with the IL-1 β /IL-6 composite genotype than for those without the composite genotype.

REFERENCE

1. Kornman, KS, et al. Ann Periodontol 1998; 3:327-38.
2. Fishman, D, et al. J Clin Invest 1998; 102:1369-76.