The impact of genetic variability and smoking habits on the prevalence of periodontitis among adults

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Abstract
Aim: Elucidate the effect of genetic variance of inflammatory mediators expression, the influence of microbial expression, and smoking as a risk factors for periodontitis. **Material &Methods**: Sample of this study composed of 50 smokers & 50 non smoker volunteers (unrelated and of the same ethnic population) with 40-60 years old .Their periodontal status was estimated through periodontal examination (full mouth clinical attachment loss measurement ,probing depths ,plaque index scores, and bleeding on probing). Isolation and detection of certain oral pathogens; *A.actinomycetemcomitans* , *Porphyromonas gingivalis* ,*and Provetella intermedia* was performed . Genotype for bi-allelic IL-1A+4845, IL-lB+3954 gene polymorphisms using mouth wash was detected by PCR based methods. **Results**: There were a significant difference only between the two groups (smokers &non-smokers) as regards to colonization of *A.actinomycetemcomitans* & not among *Porphyromonas gingivalis* & *Prevotella spp*. There were no significant difference between the overall frequencies of carrying allele 2 of IL-l A, IL-1B among smoker and non-smokers. The percentage of non smokers having healthy periodontal status was much higher than smokers and the difference was significant. On the other hand, smokers recorded much higher percentage for mild,moderate and severe periodontitis.The difference was statistically significant concerning the percentage of those with severe periodontitis. **Conclusion:** Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-l A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated *A.actinomycetemcomitans* growth.
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Key words: genetic polymorphism, periodontitis, Interleukin -1, periodontal pathogens, smoking

**** Abbreviations:**
A.actinomycetemcomitans =A.actino.
CAL = Clinical Attachment loss
Interleukin -1 = IL-1
Porphyromonas gingivalis= P. Gingivalis
Provetella intermedia =P. intermedia.

Introduction:

The oral cavity is vulnerable to external agents as cigarette smoking exposure which causes oral changes in both hard and soft tissues *Susin,et al.,(2004).*

Periodontitis is a chronic inflammatory disease initiated by specific bacteria that activate host mechanisms destroying bone and connective tissues which support the teeth. Substantial data supported the current concept that specific bacteria are essential for initiation and progression of chronic periodontitis (*Page et al., 1997*), but the rate of progression and disease severity are determined by host modifiers such as smoking *(Bergstrom, 1989*),diabetes, (*Collin et al., 1998)* and genetic influences.(*Kornman,2006)*

Smoking has major effects on the host response, but there are also a number of studies recorded some microbiological differences between smokers and non-smokers (*Gomes ,et al., 2006).*

The pro-inflammatory cytokine interleukin-1 (IL-1) is a key regulator of the host responses to microbial infection and a major modulator of extracellular matrix catabolism and bone desorption. It has been reported that variations in the IL-1 gene cluster on chromosome 2 are associated with increased susceptibility to severe periodontitis (*Mc Devitt, et al., 2002).*Therefore, a genetic test was being marketed to predict risk for periodontal disease progression *(Higashi ,2002).*

The aim of this study Elucidate the effect of genetic variability including the variance of inflammatory mediators expression ,the influence of microbial expression, and smoking as effects influencing risk for periodontitis.

Materials and Methods:

Subjects:

Sample of this study composed of 50 smokers & 50 non smokers volunteers (unrelated and of the same ethnic population) with 40-60 years old. Both groups were interviewed and filled a detailed questionnaires for family history, dental, medical as well as smoking habits,. Cigarette consumption was calculated (i.e. mean numbers of packs/day× number of years smoked)

Methods:

1-Periodontal Examination:

Criteria for assessment of the severity of periodontitis:

1- Mild periodontitis: Mean CAL ≥0.6 mm to 1.5 mm,no. interproximal sites with CAL≥3mm.No more than 3 missing teeth with the exception of orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing.
2- Moderate periodontitis: Mean CAL ≥1.6 mm to 2.4 mm and≤ 8mm, interproximal sites with CAL≥3mm distributed through at least 3 quadrants or at 6 teeth. No more than 5 missing teeth with the exception of third molars, teeth extracted for orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing
3- Severe periodontitis: Mean CAL ≥ 2.5 mm and 1 or more sites in 3 out of 4 quadrants with interproximal sites with CAL≥5mm. No more than 14 missing teeth with the exception of third molars, teeth extracted for orthodontic purpose, teeth lost as a result of extra oral trauma or extensive decay ,or teeth that were congenitally missing

2-Microbiological Examination

Sampling : Paper-point samples were taken from the 4 deepest sub-gingival sites in each quadrant of the dentition (*Mombelli et al., 1991, 1994*).Samples were then placed in 0.9 ml of sterile pre-reduced anaerobically transport fluid(RTF) (*Loesche eta;., 1972*) and transferred to the laboratory within 10 minutes.

Culturing: The samples were dispersed for 6 sec. with a vortex mixer and 10 fold serially diluted in RTF. Aliquots of 0.1 ml of appropriate dilutions were placed in duplicate onto specific media for different microorganisms in concern.

Samples were grown anaerobically (80% N2, 10% H2, 10% C02) at 37°C on 5% horse blood agar plates (Oxoid no. 2, Basingstoke, England) enriched with hemin (5 mg/L) and menadione (1 mg/L) for detection of *Porphyromonas gingivalis* and on Trypticase soyserum bacitracin-vancomysin (TSBV) medium in air with 5%CO2 at 37°C for the selective isolation of A.*actinomycetemcomitans*. KVLB-2 (kanamycin 75μ g/ml–Vancomycin 2 μ g/ml laked blood agar) for isolation of pigmented and non pigmented *Prevotella spp*.

Identification*: Porphyromonas gingivalis* was identified on the basis of Gram stain, anaerobic growth, and the inability to ferment glucose, the production of indole, and a positive hemagglutination test with 3% sheep erythrocytes.

A.actinomycetemcomitans was identified on TSBV plates, based on typical colony morphology and positive catalase reaction. The percentage of the microorganisms of total colony-forming units (CFU) was counted on blood agar plates.

3- PCR based methods:

a-DNA Isolation from Mouthwash

DNA from all subjects was isolated according to the method of *de Vries et al. (1996)* as modified and validated for the study of cytokine gene polymorphisms (*Laine et al., 2000).* In short, each individual rinsed out his/her mouth with 10 mL of 0.9% saline for 60 sec. Buccal epithelial cells were centrifuged at 300 x g for 10 min. The pellet was washed twice in 0.9% saline, re-suspended in 100 p1L of 50 mM NaOH,

and boiled for 10 min. Samples were neutralized with 14 pL of 1 M Tris (pH 7.5) and centrifuged at 14,000 x g for 3 min. Supernatants were collected and stored at 4°C until analysis.

b-- **Analysis of Polymorphisms in Genes of the IL- 1 A &B**

The bi-allelic polymorphisms at position -889 within the promoter region of the IL-IA gene (McDowell et al., 1995) and at position +3954 (Taq I RFLP) within exon 5 of the IL-1B gene (Bioque et al., 1995), were determined according to previously described methods.

Results :

The distribution of isolated microorganisms among examined groups was illustrated in table (1). *Porphyromonas gingivalis* , *Prevotella spp* and *A.actinomycetemcomitans* colonized (24% ,16%, and 30% of the smokers and 12% , 8% and 6% of non- smokers respectively. There were significant difference between the two groups (smokers &non-smokers) as regards to colonization of Gram (-ve) facultative rods(***A. actinomycetemcomitans)***& no significant difference between the other two organisms.

The percentage of smokers & non smokers carried allele 2 of IL-1A was more than those carried IL-1B (30%,28%& 10%,0%) respectively, table (2) . The difference was statistically non- significant. All individuals were heterozygous, except 4 were homozygous of whom carried Allele 2 of IL-1A(+4845) polymorphism .

Smokers and non- smoker individuals carried allele 2 of IL-1A and IL-1 B were further divided according to the severity of the periodontitis . The percentage of non smokers having healthy periodontal status (47%) was much higher compared to smokers and the difference was significant. On the other hand, smokers & non smokers recorded nearly a similar percentage among those complained from mild, moderate (15%, 20% & 11.8%,11.8%)with no statistical significant difference .Whereas, statistically significant difference was noted concerning the percentage of individuals in both groups complained from severe periodontitis (65%&29.4% respectively), table (3).

TABLES

Table (1): Percentage of bacterial species in plaque samples of smoker and non smoker individuals examined

Bacterial species	*% of Non Smoker individuals*	*% of smokers individuals*	*Z.score*	*P*
Gram (-) facultative rods ***A. actinomycetemcomitans***	6% (3/50)	30% (15/50)*	1.96*	0.02
Gram (-) anaerobes ***Porphyrmonas gingivalis***	12% (6/50)	24% (12/50)	1.3	0.09
Gram (-)anaerobes ***Provotella intermedia***	8% (4/50)	16% (8/50)	0.92	0.17

Table (2): Distribution of composite IL-1 genotype of allele 2 carriage of IL-1A(+4845) & IL-1B (+3953)among samples of smokers and non smokers

Studied samples	*IL-1 genotype of allele 2 carriage of Il-1A(+4845) &IL-1B(+3953)*		
	Il-1A	**IL-1B**	**Total carriers of allele 2**
Non smokers	14/50(28%)	3/50(6%)	17/50(34%)
Smokers	15/50 (30)	5/50(10%)	20/50(40%)
Total studied	29/100 (29%)	8/100(8%)	37/100(37%)

P value ;0.7

Table(3): Distribution of allele 2 frequency of IL-1A(+4845) & IL-1B (+3953)composite genotype among studied individuals in relation to severity of periodontitis

Periodontital status	Carriers of *of allele 2 of IL-1A & IL-1 of allele*			
	Non smokers (n=17)	Smokers N=20)	Z score	P.value
Healthy	(8/17) 47%*	0/20(0%)	3.06	0.001
Mild	(2/17) 11.8%	15%(3/20)	0.2	0.42
Moderate	(2/17) 11.8%	20%(4/20)	0.23	0.4
Severe	(5/17) 29.4%	65%(13/20)*	1.82	0.03

Discussion:

Periodontitis is a multifactorial chronic inflammatory disease. However, it is difficult to acertain the role of the different factors involved in its pathogenesis. Cigarette smoking is associated with increased prevalence and severity of destructive periodontitis in terms of periodontal pocketing, periodontal bone loss, and tooth loss *(Gomes,2006).*

Microbiological diagnosis, focused on a number of microbial species *e.g., Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis,* and *Prevotella intermedia*) .These microorganisms were have been proposed to be a useful tool for the identification of susceptible individuals (*Slots and Listgarten, 1988, Maiden et al., 1990 and haffajee et al., 1994).* Substantial data support the current concept that specific bacteria are essential for initiation and progression of chronic periodontitis (*Page et al., 1997*), but the rate of progression and disease severity are determined by host modifiers such as smoking, diabetes, and genetic influences.

In the present study approximately half of the smokers and third of the non smokers harbor these three microorganisms in their oral cavity. There were significant difference between the two groups (smokers &non-smokers) as regards to colonization of Gram (-ve) facultative rods (***A.*** *actinomycetemcomitans)&* no significant difference between the other two organisms.

Genes who encode inflammatory cytokines are subject to polymorphisms in their regulatory regions that may affect both the level and ratio of cytokines produced in response to exogenous stimuli. These variant alleles are observed in a large percent of the population and are often associated with increased or decreased susceptibility or severity (modifiers) to infectious, immune or inflammatory diseases (*yucesoy et al.,(2003*).

Axelsson (2002) reported that two factors, smoking and IL-1 genotype, significantly increased the risk of progression of alveolar bone loss and tooth loss due to progressive periodontitis. Moreover, the effect was synergistic: 41% of the IL-1 genotype-positive smokers lost 2 teeth, compared with roughly 11% of those who had only one of the risk factors.

Our results correlated the severity of periodontitis to presence of carriers of allele 2 genotype in the IL-1A and IL-1B genes. A data agreed with *Kornman et al., (1997*) who reported the same correlation and explained this finding as genetic mechanism by which some individuals, if challenged by bacterial accumulations ,may have more vigorous immune-inflammatory response leading to more severe periodontitis..Moreover, *Kornman(2006)* added that monocytes from individuals homozygous for the IL-1 B +3953 allele 2produce four-fold more IL-1 β and heterozygous cells produce approximately two-folds more IL-1 β from individuals homozygous for allele1.

Our results showed that the nature of the host response is determined primarily by genetic factors , environmental and acquired factors (smoking).

The complex interactions that occur between host-response mechanisms and oral pathogens in periodontal disease have made elucidation of genetic factors in disease susceptibility more difficult *(Hassell et al., 1995).*

C**onclusion :** Environmental factors play either a direct (i.e., causative factor) or indirect (modifying factor) role as a risk factor for periodontitis. The association between genetic polymorphism of allele 2 of IL-l A, IL-1B expression & smoking habits caused a synergistic effect for progression of periodontitis. Smoking initiated *A.actinomycetemcomitans* growth.

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