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College of Dentistry

The Association of Cytokines (TNF-α, IL-10) and HLA class I (A and B) Genes Polymorphisms with Chronic Periodontitis

A Thesis

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Abstract

Background: Chronic periodontitis (CP) is an inflammatory disease of bacterial etiology that causes damage to the supporting tissues of tooth. The inflammatory response of these tissues to infection is influenced by environmental and genetic factors. The human leukocyte antigens (HLA) play a significant role in immune responsiveness and involve in antigen recognition of periodontal pathogens. The human leukocyte antigens polymorphisms could represent an important susceptibility or resistance factor to periodontitis. Furthermore, genetic polymorphism in cytokine genes is regarded as a promising factor in inducing periodontitis. Tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) are cytokines that have complex and opposing roles in the inflammatory responses. The polymorphism at position (-308) of tumor necrosis factor-α and position (-1082) of interleukin-10 genes have been reported to influence the expression of tumor necrosis factor-α and interleukin-10 respectively, thereby playing a role in the pathogenesis of periodontitis.

Aims of the Study: This study was performed to investigate the differences in allele frequencies of human leukocyte antigens class I (HLA-A and HLA-B) in patients with chronic periodontitis compared with control group. Also to study the genetic susceptibility in the patients by genotyping of HLA-A and HLA-B alleles and cytokines (Tumor necrosis factor-α and Interleukin-10) genes and to find out whether any association exists between these gene polymorphisms and the severity of chronic periodontitis.

Materials and Methods: Fifty subjects with chronic periodontitis (30 males and 20 females) with an age range of 30-50 years and 20 healthy volunteers with clinically healthy periodontium (10 males and 10 females) with an age range of (25-45) years were participated in this study. Plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level were the periodontal parameters that used in this study. Patients were classified

into three subgroups according to the severity of clinical attachment loss (mild, moderate and severe chronic periodontitis). Five ml of venous blood was withdrawn from all subjects that participated in this study and then DNA was extracted from blood samples. HLA-A and HLA-B genotyping was done by polymerase chain reaction-sequence specific oligonucleotide probes. Fifty samples only (35 patients and 15 control) were analyzed for polymorphism of tumor necrosis factor-α gene at position (-308) and interleukin-10 gene at position (-1082). The results of electrophoresis of PCR products for these cytokines were undergone for sequencing to locate the positions of possible mutations.

Results: Data revealed that the frequency of HLA-A*33 was significantly higher in patients than in controls (P= 0.0268). The comparison between each subgroup of patients and controls, revealed that the frequency of HLA-A*23 allele was significantly higher in severe group (p=0.047), and HLA-A*33 allele frequency in moderate chronic periodontitis remained significantly higher than control group. On the other hand, there was significant increase in the frequencies of HLA-B*27 and HLA-B*51 alleles in control group than that in patients group. Interestingly, the current study also showed that the frequency of HLA-B*51 allele remained significantly higher in healthy control as compared to severe group and moderate group patients. The results of sequencing for tumor necrosis factor-α and of interleukin-10 genes showed higher frequency of mutationsin patients as compared to control samples. In addition the results revealed a highly significant difference in the frequencies of mutations among the six samples that undergone sequencing (4 patients and 2 controls), (P= 0.0002).

Conclusion: This study demonstrates that HLA-A*33 allele may contribute to increased susceptibility to chronic periodontitis. Whereas HLA-B*27 and HLA-B*51 alleles could be considered as protective factors for the chronic

periodontitis disease. Moreover, the current results suggests that the (-308) polymorphism in tumor necrosis factor-α gene and (-1082) polymorphism ininterleukin-10 gene may be associated with the susceptibility to chronic periodontitis.