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**Analysis of Nuclear Factor Kappa and Toll-Like
Receptor-4 Genes Polymorphisms and there
Associations with Selected Immunological
Biomarkers in Iraqi Population with severe Chronic
Periodontitis**

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Abstract

Background: There is a strong evidence to support that periodontitis is an inheritable disease and cytokines can signal and amplify the immune responses during infection. However, chronic inflammation with aggravated production of cytokines results in the destruction of periodontal tissue. Furthermore, a number of genetic polymorphisms have been associated with risk for periodontal disease development in various populations. It is well known that genetic association function essential role on susceptibility to periodontal disease, but the genetic markers correlated to periodontal disease have not been fully clarified. The genes encoding toll like receptor-4 and nuclear factor- kappa B may impact susceptibility and/or severity of periodontitis.

Aims of the Study: understanding the potential contribution of genetic variation in toll like receptor-4 and nuclear factor- kappa B on periodontitis, the current study used a case control approach to explore the correlation between polymorphisms in these genes with the degree of susceptibility and severity of chronic periodontitis and its role on the serum level of some inflammatory cytokine, interleukin -8 and interleukin -18.

Materials and Methods: Ninety six Iraqi subjects (69 males and 27 females) with age range (30-50) years were included in this study. They were divided in to two groups (55 patients with severe chronic periodontitis and 41 healthy controls). The clinical periodontal parameters (plaque index, gingival index, probing pocket depth, clinical attachment loss and bleeding on probing index) were recorded and then 5ml of venous blood collected from each subject for immunological and genetic analysis. Detection of serum cytokines levels were done by the use of enzyme -linked immunosorbant assay kits then genetic analysis of toll like receptor-4 and nuclear factor- kappa B including Deoxyribonucleic acid extraction, polymerase chain reaction amplification and sequencing by sanger method were done for each subject in both groups .

Results: The present study revealed a significant decrease in the median serum level of interleukin -8 and interleukin -18 at p-value < 0.05 among chronic periodontitis group as compared to control group. Moreover, the current work illustrated significant negative correlation between interleukin -8 and each of plaque index, gingival index and bleeding on probing in chronic periodontitis group while interleukin -18 was positively correlated with probing pocket depth.

Concerning the results of genetic analysis, two single nucleotide polymorphisms (SNPs) were detected in toll-like receptor -4 according to primer design located at rs 4986790 and rs 4986791 in exon 4. Analysis by Hardy-Weinberg equilibrium for study groups were done for the two SNPs, the results were significant in control group and total sample for rs 4986790. While non-significant result of equilibrium were obtained regarding rs 4986791. On the other hand, the SNPs number and distribution was one SNP in chronic periodontitis group and 3 SNPs in control group with non-significant differences between the groups. Furthermore, there was 4 SNPs in both chronic periodontitis group and control group at rs 4986791 with non-significant difference between them. Interestingly, both SNP were strongly correlated with the amount of dental plaque. Regarding the genetic analysis of nuclear factor-kappa B1-94, two polymorphisms were detected in the promoter region at rs28362491 and rs569599236 with non-significant difference between groups. Although there were 30 polymorphisms for rs28362491 in chronic periodontitis group and 23 in control group, while there was only one polymorphism for rs 569599236 in chronic periodontitis group. There was a significant correlation of interleukin -8 and interleukin -18 with rs28362491 as well as with bleeding on probing index in chronic periodontitis group.

Conclusions: Genetic polymorphisms in toll like receptor -4 and nuclear factor-kappa B1-94 ins/del ATTG were not associated with the susceptibility of severe periodontitis in Iraqi patients. However, they might have an association with the

clinical periodontal parameters and can modify severity of the disease. High frequencies of toll like receptor-4 at rs4986790 in control group suggest that these alleles may confer protective effects against the disease or weak susceptibility to periodontal disease.