# Association of TGF –beta1 gene polymorphism with chronic periodontitis.

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## Abstract

Back ground: TGF-β1 is present during both early and late stages of periodontitis,among individuals the TGF-β1 varies from person to person depending on TGF-β1 gene polymorphisms.Aim of study: is to association of TGF-β1gene polymorphism with chronic periodontitis by sequencing technique. Material and Method: A total of 75 sample, 50 patient of chronic periodontitis (cp) and 25 normal subject, referring to the clinic of collage of Dentistry, University of Babylon, Hilla City, Iraq. direct sequencing of 786pb of exon1 Result: The results of sequencing showed five (SNPs)two are known SNPs rs1800470..A260 and rs11466316..G675A and three a three of them are novel g.14112183C.T..C301T, g.14112467G.C..G585C, g.14112568G.T..G686T. the statistical analysis reveal that there is a significant association between rs1800470 polymorphism with chronic periodontitis while the other polymorphisms did not record a significant association. Conclusion: The present study suggest that there is significan t between TGF-β1 gene (rs1800470..A260)polymorphism and chronic periodontitis.

**Key world: periodontal disease, TGF-β1, Chronic periodontitis,**

## 1.Introduction

Periodontal disease (PDs) is amultifactorial inflammatory disease affecting the population (Vos *et al*., 2012). Periodontal disease are inflammatory lesions initiate by oral bacteria that lead to the destruction of the supporting structures of the teeth (gingiva, periodontal ligaments and alveolar bone ) ( Wolf and Lamster, 2011).

Transforming growth factor beta 1 (TGF-β1) is a multifunctional cytokine ,that has a regulatory role in a broad spectrum of cellular processes such as differentiation, growth, apoptosis, immune reactions and angiogenesis(Hanafy and Ado, 2011).

TGF-β1 is involved in the synthesis of connective tissue components including collagen, glycosaminoglycan, proteoglycan, fibronectin and osteonectin, in cells of periodontal ligament and many others cell types . (Okada and Murakami, 1998 ; Huang *et al*., 2015). TGF-β1 production

differs between peoples , depending on the TGF-β1 gene polymorphisms.The human gene encoding TGF-β1 which is located on chromosome 19q13.1–13.3 (Eliopoulos et al.,2009). Among the three homologous isoforms present in mammals (TGF-1, TGF-2, TGF-3), TGF-1 is the most abundant and ubiquitously expressed isoform (Derynck *et al*., 2001; Kubiczkova *et al*., 2012).

Holla et al showed in their study among sample in Czech population , the possible association of five polymorphisms of the TGF-beta1 gene { (3 in the 5 region at positions –988 (C/A),800 (G/A),509 (C/T) and two located at codons 10 (L10P) and 25 (R25P) of exon 1)}; and its association with chronic periodontitis and found that there were non-significant differences in all of these five polymorphisms.(Holla *et al*., 2002)

De souza et al showed in their study among sample in Brazilian population to investigation the possible association of TGF beta1{(-509C/T)} gene polymorphism with chronic periodontitis (moderate and sever),and was showed significant of TGF beta1{(-509C/T)} gene polymorphism with chronic periodontitis.(De souza *et al*., 2003).

Babel et.al. showed in their study in Germany population the association of TGF beta1 codons 10 (L10P) and 25 (R25P) of exon 1) polymorphisms with chronic periodontitis and founded that there was a significant association in TGF beta1(codon 25) ,while in codon 10 was non-significant association with chronic periodontitis. (Babel *et al*., 2006 )

Atilla et al evaluated TGF-β1 +915G/C , Thr263Ile and 713/8delC gene polymorphisms in a Turkish population with chronic and aggressive periodontitis and found that there's was association between TGF-β1 genotype and chronic periodontal disease(Atilla *et al*., 2006)

Atanasovska-Stojanovska et al showed in their study in Macedonian population analysis of TGF-β1{(codon 10/C:T)},and {(codon 25/ G:G)} gene polymorphism with chronic periodontitis and founded that (TGF-β1 cdn10/T, and, TGF-β1cdn25/G alleles) were associated negatively with periodontitis. The accumulative effects of cytosine (C) position in TGF-β1 cdn10 changed from the lowest in TGF-β1 cdn10/C allele, bigger in TGF-β1 cdn10/C:C genotype; almost doubled in TGF- β1 cdn10/CC haplotype and biggest in TGF-β1 cdn10/CC:CG haplotype combination.( Atanasovska-Stojanovska *et al*., 2009).

Heidari et al, in their study among sample of Iranian population,studied the probable relationship

p between TGF-β1 −509 C/T (rs1800469), 29 C/T(Prol10Leu, rs1800470) and 788 C/T (Thr263Ile, rs1800472) gene polymorphisms and chronic periodontitis ,and founded that the TGF-β1 29 C/T polymorphism was significant association with chronic periodontitis , but −509 C/T and 788 C/T polymorphisms, may not associated with the development of chronic periodontitis.( Heidari *et al*., 2013).

## 2. Material and methods

**2.1 .Sample selection**:- The subjects registered in the present study composed of (75) subjects of both genders. The age range from (25-62) years. Most of the subjects were from attendants to department of Periodontics; College of Dentistry , University of Babylon, Hilla city, Iraq, between October 2017 and February 2018 .Control group (C)included(25) subjects who had healthy periodontium Chronic periodontitis group(CP) :- included (50) subjects with chronic periodontitis ( 30% of teeth with clinical attachment level ≥ 5mm)were considered severe (Kornman et al 1997).The disease severity were based on criteria established in 1999 at the International Workshop for a classification of periodontal disease and conditions. (Armitage.,1999)

### 2.2 DNA extraction

**Blood collection**: Under sterile conditions 5ml of the venous blood was withdrawn of the patient Blood sample was collected into (EDTA tubes) Extract DNA from frozen samples of blood by use Favorgen kit, Taiwan.

**Saliva collection**: Under sterile condition Epithelial buccal cells were collected from mouthwash was performed with 5 ml of 3% glucose for 1 minute Extract DNA from frozen samples of saliva by use Favorgen kit, Taiwan. (Trevilatto and Line 2000).

### 2.3 PCR Amplification and PCR Amplicons Sequencing of TGF beta1.

One PCR fragment was selected for amplification, which supposed to cover 786 bp of the exon 1 of transforming growth factor, beta 1 (TGF B1)gene.The sequence of the FP is 5′-CCCAGACCGCCTCCCTTTG -3′, while the sequence of the RP is 5′-CTCCGGTTCTGCACTCTCC -3′ . The PCR program on a thermal cycler in TGFB1was: a first denaturation step. At (94°C)for (5 min), followed. By (35cycles) of (94°C) for (40s), (65 °C) for (30s), (72 °C) for (1min), and a final extension0step of (5min) at (72°C). 5 µl of the amplification productswas electrophoreses on a(2%) agarose gel at(150 V) for (90 min).It was made sure that all

PCR resolved bands are specific and consisted of only one clean and sharp bands before being submitted to sequencing experiments.The 786 bp PCR amplicons were commercially sequenced from both ends according to instruction manuals of the sequencing company (Macrogen Inc. Geumchen,South Korea). Only clear chromatographs obtained from ABI sequence files were further analyzed, ensuring that the annotation and variations are not because of PCR or sequencing artifacts. By comparing the observed DNA sequences of both local specimens with the retrieved DNA sequences of *TGFB1* locus (GenBank acc. LM651059.1), the exact position and other details of the retrieved PCR fragment was identified Figure (2.1).

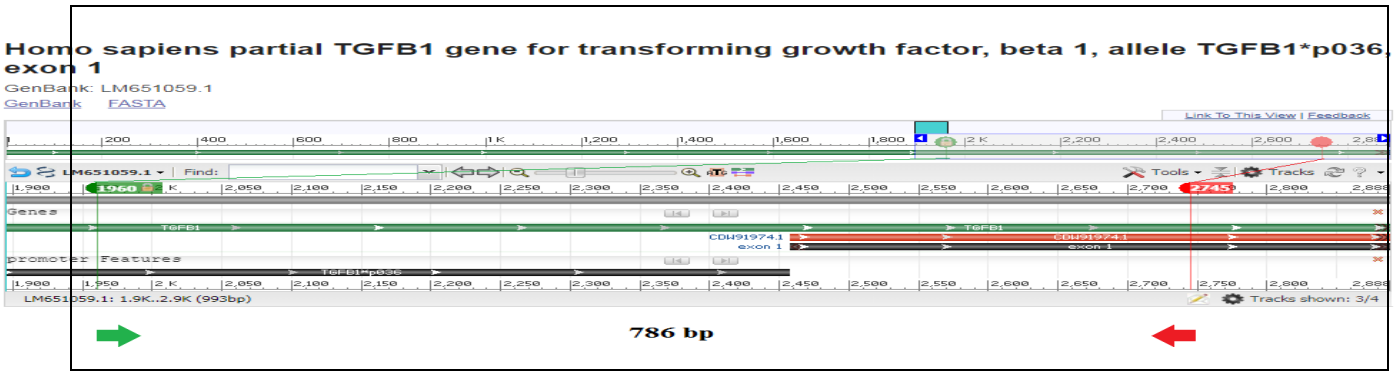



**Figure (2.1)** The exact position of the studied 786 bp amplicon within *TGFB1* referring sequences (GenBank acc No. LM651059.1). The green arrow refers to the starting point of this amplicon while the red arrow refers to its end point.

## 3. Result

### 3.1: TGF-β1Genotyping PCR

PCR product of TGF-β1 gene was amplified by specific primer the PCR product (band) was 786 pb , before PCR product sequencing that showed in figure (3.1).

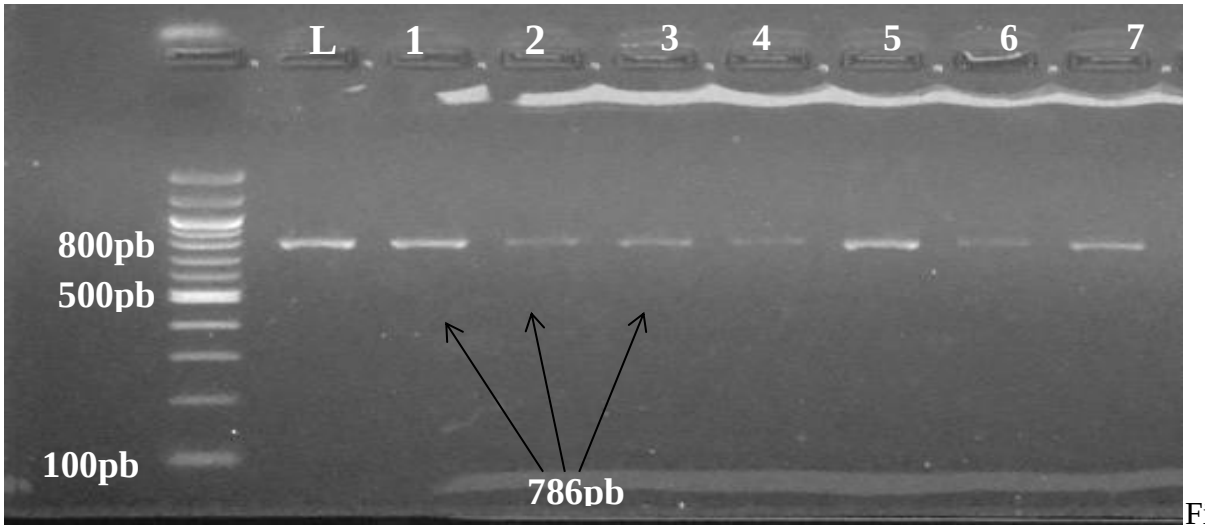


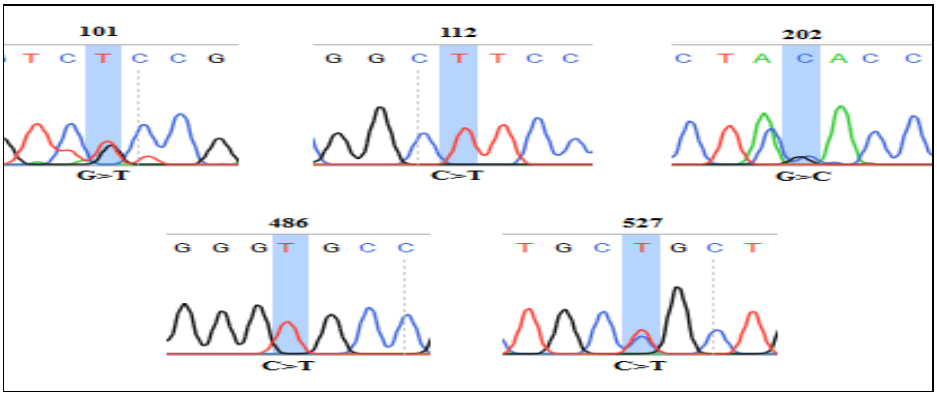
Figure (3.1)electrophoresis pattern of PCR product of TGF-β1 gene, PCR product 786pb. L: ladder DNA.

### 3.2 DNA sequences alignment of the 786 bp amplicon of the TGF-β1 sequences (1 – 786 nucleotides range).

DNA sequences alignment of the observed variants with its corresponding reference sequences of the 786 bp amplicon of the TGF-β1 sequences (1 – 786 nucleotides range).The alignment results of the obtained SNPs were clarified as a whole in one comprehensive.

### 3.3 The alignment results of all sequencing samples

The alignment results of all sequenced samples revealed the presence of five SNPs, in which five substitution mutations were observed. The sequencing chromatogram of the observed SNP is documented in amplicon figure (3.2).



Figure(3.2) The pattern of DNA chromatogram of the 786 bp amplicon of the *TGF-B1* amplified locus. The observed substitution SNP was highlighted according to its position in the PCR products. The symbol ">" refers to a substitution mutation.

### 3.4.1 Allelic association of TGF-β1 gene polymorphism.

Allelic association of TGF-β1 gene polymorphism for case and control are listed in Tables (3.1) The results showed that there was non-significant of allelic frequency difference between case and control groups in all SNPs.

**Table(3.1) Allele association of TGF-β1 gene polymorphism**

| **rs1800470** | Control | | Case | | OR (95% CI) | P-value* |
|---|---|---|---|---|---|---|
| Allele | Count | Proportion | Count | Proportion | | |
| A | 39 | 0.78 | 73 | 0.73 | 0.763 (0.342-1.700) | 0.50 |
| G | 11 | 0.22 | 27 | 0.27 | 1.311 (0.588-2.923) | |
| **rs11466316** | | | | | | |
| G | 50 | 1 | 99 | 0.99 | 0.657 (0.026-16.413) | **1.00** |
| A | 0 | 0 | 1 | 0.01 | 1.523 (0.061-38.050) | |
| **g.14112183C.T** | | | | | | |
| C | 50 | 1 | 98 | 0.98 | 0.390 (0.018-8.280) | 0.57 |
| T | 0 | 0 | 2 | 0.02 | 2.563 (0.121-54.413) | |
| **g.14112467G.C** | | | | | | |
| G | 49 | 0.98 | 100 | 1 | 6.091 (0.244-152.239) | 0.42 |
| C | 1 | 0.02 | 0 | 0 | 0.164 (0.007-4.104) | |
| **g.14112568G.T** | | | | | | |
| **G** | **49** | **0.98** | **96** | **0.96** | | |
| T | 1 | 0.02 | 4 | 0.04 | 2.042 (0.222-18.764) | **0.66** |

*Two tailed p value of Fisher exact test .

### 3.4.2 genotyping association of TGF-β1 gene polymorphism.

Genotyping association of TGF-β1 gene polymorphism for case and control are listed in Tables (3.2). The results presented that there was non-significant of genotyping frequency difference between case and control groups in all SNPs.

**Table(3.2) genotyping association of TGF-β1 gene polymorphism .**

| **(rs1800470)** | | | | |
|---|---|---|---|---|
| **Genotype** | **Control** | **Case** | **OR (95% CI)** | **P-value*** |
| **A/A** | **14 (56%)** | **23 (46%)** | **1.00** | **0.41** |
| **A/G** | **11 (44%)** | **27 (54%)** | **1.49 (0.57-3.93)** | |
| **rs11466316** | | | | |
| **G/G** | **25 (100%)** | **49 (98%)** | **1.00** | |

| | | | | |
|---|---|---|---|---|
| **G/A** | **0 (0%)** | **1 (2%)** | **1.54(0.061-39.306)** | **0.37** |
| **Novel SNPs** | | | | |
| **g.14112183C.T** | | | | |
| C/C | 25 (100%) | 48 (96%) | 1.00 | 0.2 |
| C/T | 0 (0%) | 2 (4%) | 2.629 (0.122-56.857) | |
| **g.14112467G.C** | | | | |
| G/G | 24 (96%) | 50 (100%) | 1.00 | 0.14 |
| G/C | 1 (4%) | 0 (0%) | 0.162 (0.006-4.116) | |
| **g.14112568G.T** | | | | |
| **G/G** | **24 (96%)** | **46 (92%)** | **1.00** | **0.5** |
| **G/T** | **1 (4%)** | **4 (8%)** | **2.09 (0.22-19.73)** | |

***Two tailed p value of chi square

### 3.4.3 Hardy-Weinberg equilibrium exact test association of TGF-β1 gene polymorphism.

Hardy-Weinberg equilibrium exact test showed that there was highly significant (P=0.01) deviation in case group only in (rs1800470) SNPs. while the other SNPs did not record a significant association As shown in Table (3.3).

**Table(3.3) Hardy-Weinberg equilibrium association of TGF-β1 gene polymorphism**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Recorder SNPs** | | | | | | |
| **rs1800470** | **AA** | **AG** | **GG** | **A** | **G** | **P-value*** |
| **All subjects** | 37 | 38 | 0 | 112 | 38 | **0.002** |
| **Control** | 14 | 11 | 0 | 39 | 11 | 0.3 |
| **Case** | 23 | 27 | 0 | 73 | 27 | **0.01** |
| **rs11466316** | GG | GA | AA | G | A | |
| **All subjects** | 74 | 1 | 0 | 149 | 1 | **1** |
| **Control** | 25 | 0 | 0 | 50 | 0 | **1** |
| **Case** | 49 | 1 | 0 | 99 | 1 | **1** |
| **Novel SNPs** | | | | | | |
| **g.14112183C.T** | CC | CT | TT | C | T | |
| **All subjects** | 73 | 2 | 0 | 148 | 2 | **1** |
| **Control** | 25 | 0 | 0 | 50 | 0 | **1** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Case** | 48 | 2 | 0 | 98 | 2 | **1** |
| **g.14112467G.C** | GG | GC | CC | G | C | |
| **All subjects** | 74 | 1 | 0 | 149 | 1 | **1** |
| **Control** | 24 | 1 | 0 | 49 | 1 | **1** |
| **Case** | 50 | 0 | 0 | 100 | 0 | **1** |
| **g.14112568G.T** | GG | GT | TT | G | T | |
| **All subjects** | 70 | 5 | 0 | 145 | 5 | **1** |
| **Control** | 24 | 1 | 0 | 49 | 1 | **1** |
| **Case** | 46 | 4 | 0 | 96 | 4 | **1** |

*Two tailed p value of Fisher exact test

## 4. Discussion

Periodontal disease is affecting the supportive tissues,which surround the teeth. The incidence of periodontal disease is somewhat high among population and when left without treatment may lead to tooth loss (Albandar 2005).

Transforming growth factor-Beta1 (TGF-β1) is a cytokine ,which considered as multifunctional,and regulates a change of cellular processes including immune reactions ,growth, differentiation, apoptosis, and ontogenesis (Hanafy and rumpus, 2011) . Our study is the first study that showed the TGF gene polymorphisms association with the chronic periodontitis by sequencing technique among 75 subjects of Iraqian population in Hilla province. Sequencing technique is very important technique in genetic study because its discoverd novel SNPs and large number of SNPs in the studying gene.

Sequencing technique is to investigate of the single nucleotide polymorphisms(SNPs) in target area from the gene and study association of (SNPs) with the disease; and the target area of this study is part promoter and also part of exon 1 of TGF beta1 gene that related with chronic periodontitis.

The results of this study of sequencing are five single nucleotide polymorphisms(SNPs) in this part of gene .Two (SNPs) had been recorded before hand in National Center Biotechnology Information (NCBI) and three (SNPs)were novel.

The association of TGF-β1(rs1800470)gene polymorphism with chronic periodontitis was statistically significant between case and control groups.This result was accordance with previous

study by Aneta Atanasovska-Stojanovska et al in Macedonian population sample ;which consisted of 301control individuals, and 132 individuals with chronic periodontitis.And they found a statistically significant association of TGF-β1 gene polymorphism{cdn10 (ref SNP ID rs1800470, C/T,, Pro/ Leu)} with chronic periodontitis between these two groups. (Aneta Atanasovska-Stojanovska et al 2009).

In addition ;this result was also accordance with the result of previous study among Iranian population sample by Zahra et al,which studied the association of 29C/T(Prol10Leu;rs1800470) and chronic periodontitis , and has showed that there was a significant difference between patient and control samples. (Heidariy et al 2013). At the same time ; The result of our study was disagreed with the results showed by Holla et al ;which studied the association of TGF-β1 gene polymorphism of codons10 (L10P) with chronic periodontitis; and found that there was statistically non significant difference between patients and healthy group (Holla et al 2002).

In addition; this our result was disagreed with previous study result by Babel et al ,which studied the association of TGF-β1gene polymorphism (codons 10 )with chronic periodontitis;and the sample were collected among Germany population and found that there's statistically non significant difference between patients and healthy group.(Babel et al 2006).

The association of this SNP of TGF-β1 gene with chronic periodontitis; was statistically non significant with chronic periodontitis.

This is the first study that investigation the association of this SNP on chronic periodontitis,and there was no previous study of this SNP.This result may be due to the small sample size when compared with other previous studies.

The association of Novel SNPs(g.14112183C.T..C301T, g.14112467G.C..G585C, g.14112568G.T..G686T) of TGF-β1 gene with chronic periodontitis; was statistically non significant with chronic periodontitis in all SNPs.This is the first study of the novel SNPs investigation the association of this SNPs with chronic periodontitis.

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