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# Identify the Interleukin-6 gene -597 A/G Polymorphism in Type 2 diabetic patients

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ABSTRACT

Tumor necrosis factor-alpha (TNFa), and interleukin-6 (IL-6) are a cytokine which has a role in a metabolic progression which might facilitate diabetes progression. The current study was aimed to investigate the presence of Interleukin-6 gene -597 A/G Polymorphism in blood samples of 64 diabetic and 30 blood samples healthy control in Al-Diwaniyah City, Iraq. Using PCR RFLP, the results have shown that IL-6 polymorphism was pronounced in the patients with type 2 diabetes, particularly the genotype AA, GG, and AG and it showed s significant association with sex and age parameter. Also, this study found that the prevalence (Carriage rates) of allele A was is much lower than that in (Carriage rates) allele G in T2DM population compared to healthy population, which is confirmed the previous results. The current data will provide a sight on the role of heterogeneity of cytokine gene in the development of T2DM. Furthermore, these cytokine genes in addition of its variants might be a potential indicator for diseases susceptibility in south Iraq.

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## INTRODUCTION

Diabetes is one of the chronic metabolic disease, which might lead to a high morbidity and mortality in adults worldwide. In 2013 only, nearly 382.000.000 people were diagnosed with diabetes mellitus (T2DM),and this number might arise to 592.000.000 by 2035 (Guariguata *et al.,* 2014). Type 2 diabetes mellitus is the most form of diabetes with around 90 % of all diagnostic cases. Over earlier decades, diabetes has transformed from a mild disease to one of the major diseases that affecting both the young and adults. In addition, the Type 2 diabetes mellitus (T2DM) was increased from 2.39% in 2000 to 5.32% in 2013 (Sharma *et al.,* 2016, Guariguata *et al.,* 2014, Saxena *et al.,* 2013). White blood cells are highly expressed Interleukins, mainly leukocytes which facilitate immune reactions and play a major role in the pathogenesis of T2DM (Zhuanping *et al.,* 2016). It has been shown that cytokines such as tumor necrosis factor-alpha and interleukin-6 have a role in the metabolic process which might mediate diabetes progression, whereas interleukin-6 (IL-6) and IL-10 impart protection. Polymorphisms in the codon region of these cytokine genes might affect on their structure as well as expression (Saxena *et al.,* 2012, Erbilgin *et al.,* 2013). Studies suggested that T2DM is correlated with a systemic inflammation as results of increase blood level of immune response of both markers and mediators. This due to the association of increase plasma IL-6 levels with patients has T2DM and also with impaired glucose tolerance, which suggests its potential role in the type 2 diabetes progression (Pradhan *et al.,* 2001, MŘller *et al.,* 2002). The high level of IL-6 in plasma is regulated by increasing of transcription and translation at the molecular level which might be affected

by existence of polymorphisms in its coding region (Pakala *et al.,* 1997). A study indicated that the single nucleotide polymorphisms (SNPs) located in IL-6 promoter might increase the risk factor of T2DM disease (Terry *et al.,* 2000). It has been noticed that increase in the expression of TNF-a in the muscle tissues led to decreases tyrosine kinase signaling, suggesting its potential role in diabetes and insulin resistance (Hotamisligil and Spiegelman, 1994, Tsiotra *et al.,* 2001). It was indicated that 75 % of IL-10 secretion is due to genetic process and its secretion is mainly controlled by the gene transcription in the nucleus. A study suggested that a combination of CA, GG and AA for TNF-a, IL-6, and IL-10 gene polymorphisms might lead to increase the susceptibility and the risk of developing T2DM (Saxena *et al.,* 2013). Therefore, this study examined the association of IL-6 gene promoter polymorphism in the association with T2DM development.

## PATIENTS AND METHODS

### Samples collections

EDTA tubes were used to collect 64 blood samples taken from patients that were diagnosed with type 2 diabetes and 30 blood samples healthy control individual from the diabetic center in Al-Diwaniya Teaching Hospital. The samples were stored in the refrigerator until use for DNA extraction.

### Genomic DNA Extraction

This study used a Genomic DNA Mini Kit, Geneaid. USA to extract Genomic DNA from frozen blood samples. The extraction was achieved by using frozen Blood extraction technique with Proteinase K according to company instructions. Next, the DNA concentration was tested by Nanodrop spectrophotometer, and then the samples placed in -20C until use for conventional PCR.

### PCR RFLP

Detection of 597 A/G polymorphism in IL-6 gene was achieved using PCR-RFLP techniques from blood samples of T2DM patients and healthy. This producer was accomplished according (Saxena *et al.,* 2014). The primers were purchased from Bioneer company, Korea) with forwarding sequences (5'-GGAGTCACA CACTCCACCT-3) and reverse sequence (5'-CTTAATAAGGTTTCC AATCAG-3). The master mix was prepared according to (AccuPower® PCR PreMix kit. Bioneer. Korea) provided protocol. The PCR tubes contain a pellet which was consist of (Taq DNA polymerase 1U, Tris-HCl (pH 9.0) 10mM, KCl 30mM, stabilizer, MgCl2 1.5mM, dNTPs 250µM, and tracking dye). The preparation of master mix was achieved according to the protocol of provided kit. This achieved by adding 5µl of DNA and 1.5µl of 10pmole of each forward primer and reverse primer mixed together, and then complete the volume to the 20µl of deionizer PCR water. All the cocktail above was mixed, vortexed then placed in the thermocycler (T100 Thermal cycler Biorad. USA) and following protocol was applied; initial denaturation temperature was 94°C for 5 min; followed by 35 cycles at denaturation 94°C for 30 s, annealing was 57°C for 30 s, and extension was 72°C for 1

**Table 1: Clinical and controls characteristics & patients cases**

| Clinical characteristics | | Control (N= 30 case) | Patients (N= 64 case) | P value |
|---|---|---|---|---|
| sex | Male | 7(23.33%) | 27(42.18%) | 0.07 |
| | Female | 23(71.42%) | 37(57.81%) | |
| Age | | 32.46±2.31 | 39.81±1.16 | 0.007* |

Values of age represent mean ± standard error
* significant differences at level 5%

**Table 2: Genotype frequencies**

| Type | Groups | |
|---|---|---|
| | Controls number & count (%) (N=30) | Cases number & count (N= 64) |
| AA | 13(43.33) | 45(70.31) |
| GG | 6(20) | 7(10.93) |
| AG | 11(36.66) | 12(18.75) |
| P value | 0.042 | |

Significant differences at level 5%

**Table 3: Allele frequencies**

| Type | Groups | |
|---|---|---|
| | Controls number & count (%) (N=30) | Cases number & count (N= 64) |
| A | 37(61.66) | 102(79.68) |
| G | 23(38.33) | 26(20.31) |
| P value | 0.009 | |
| Odds ratio (95% CI) | 0.4101(0.2087 to 0.8058) | |

**Table 4: Carriage rates for allele A**

| Type | Groups | |
|---|---|---|
| | Controls number & count (%) (N=30) | Cases number & count (N= 64) |
| A(+) | 24 (80) | 57(89.06) |
| A(-) | 6 (20) | 7(10.93) |
| P value | 0.241 | |
| Odds ratio (95% CI) | 0.4912(0.1494 to 1.6150) | |

**Table 5: Carriage rates for allele G**

| Type | Groups | |
|---|---|---|
| | Controls number & count (%) (N=30) | Cases number & count (N= 64) |
| G(+) | 17(43.33) | 19(29.68) |
| G(-) | 13 (52.38) | 45(70.31) |
| P value | 0.013 | |
| Odds ratio (95% CI) | 3.097(1.2599 to 7.6137) | |

min and final extension was 72°C for 5 min. an agarose gel with 2% was used to examine the PCR products using ethidium bromide, and then visualized with UV illumination. RFLP techniques was achieved using (FokI, Biolabs, UK) restriction enzyme to detect IL-6 gene 597 A/G polymorphism; (AA) wild-type homozygote, the product digested by restriction enzyme and the band size was 527bp, the (A/G) heterozygote, the product was digested by restriction enzyme and the bands size were 527bp, 461bp, and 66bp, the (GG) mutant type homozygote, was digested by restriction enzyme and the bands size were 461bp and 66bp.

## RESULTS

The current study investigated the effects of sex on the genotypes of the IL-6 gene in the patient compared to the healthy group. The results showed that there were significant effects of the gender parameter ($p$<0.05) on the IL-6 gene polymorphism. The effects of the age parameter on the IL-6 gene polymorphism were also investigated, and the results indicate a significant ($p$<0.05) increased of the IL-6 gene polymorphism within the age in the patient group compared to healthy group table1.

The genotype frequencies of the IL-6 gene polymorphism were examined in the patient group compared to healthy control group. For the AA, GG and AG genotype, the results showed a significant ($p$<0.05) presence of these genotypes in the patient group compared to healthy control group. In addition, this study investigated the allele A and Allele G frequencies in the patient population as well as in the healthy population, and the results showed a significant shifting in the both of alleles compared to healthy group table 3. The Carriage rates for allele A and G were examined in the patient population compared to the healthy population, and no significant changes were noticed in Carriage rates for allele Aalleles. However, a significant change was noticed in Carriage Rates G alleles in the patient population compared to healthy population table 4,5.

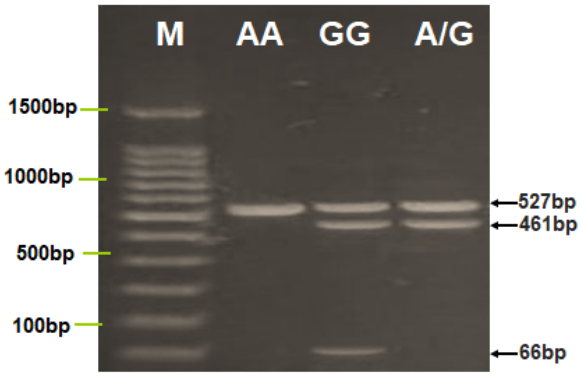


**Figure 1: Agarose gel electrophoresis image that shows the RFLP-PCR product analysis of interleukin-6 *(IL-6)*-597 A/G (rs1800797) polymorphism using FokI restriction enzyme.** Where M: marker (1500-50bp), The (AA) wild-type homozygote, the product undigested by restriction enzyme 527bp band. The (GG) mutant type homozygote that shows digested by restriction enzyme into 461bp and 66bp band. The (A/G) heterozygote, the product digested by restriction enzyme into 527bp, 461bp and 66bp band.

## DISCUSSION

The current study found that Interleukin-6 gene -597 A/G Polymorphism showed significant correlation with T2DM. This study also suggested that SNP -597 which is located in the IL-6 gene promoter region might be a risk factor to develop of T2DM. This might give a suggestion that there is a strong link between T2DM and IL-6 gene polymorphisms which might consider as a prognostic biomarker for progression of type 2 diabetes. The results from this study is reasonable because an early study has demonstrated that in addition to role of IL-6 as immune regulator, it also has a role in glucose homeostasis as well as glucose metabolism inside adipocytes, hepatocytes, skeletal muscle cells, neuroendocrine cells and pancreatic b-cells (Han

*et al.,* 2017). In addition, a meta-analysis study has supported our finding which indicated that there is a link between the genetic change of IL-6 gene with increasing risk factor to develop T2DM (Yin *et al.,* 2013). The IL-6 polymorphism was more pronounced in the patients with type 2 diabetes, particularly the genotype AA, GG, and AG and it showed s significant association with sex and age parameter. The females were showed more occurrence of this genotype of the IL-6 gene in their samples with 57.81% percentage. Therefore, IL-6-597 A/G Polymorphism that the frequency of -592 IL-10 -592 C/A polymorphism shown that the frequency A\G alleles was significantly increased in type 2 diabetes patients compared to healthy. This suggesting that this SNP has a crucial role in susceptibility to develop type 2 diabetes, which is agreed with (Chang *et al.,* 2005). Also, this study found that the prevalence (Carriage rates) of allele A (0.4912%) was is much lower than that in (Carriage rates) allele G (3.097%) in T2DM population compared to healthy population, which is confirmed the previous results. This investigational study found that AA genotype increased the risk up to 70.31% in T2DM compared to healthy group 43.33%. While for GG and AG was decreased the risk in the T2DM compared to healthy control. Also, allele A frequency increased to approximately to 79.68% in the T2DM compared to healthy. While, allele G frequency was not given any significant results (Saxena *et al.,* 2013).

## CONCLUSION

This finding suggested that that genotypes and alleles are associated with progression of type 2 diabetes, however only in combination. The current data will provide a sight on the role of heterogeneity of cytokine gene in the progression of T2DM. Furthermore, these cytokine genes in addition of its variants might be a potential indicator for diseases susceptibility in south Iraq.

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