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# Association of TNF-α genotype with Rheumatoid Arthritis patients in Iraq.

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**ABSTRACT**

Rheumatoid Arthritis (RA) has been the most disease that recorded high number in Iraqi population, so the association between some immune genes and this disease have been interested ,this study aims to investigate TNF-α gene polymorphism in Rheumatoid Arthritis patients, a case- control study included 44 patients ( from Hilla city) and 33 control blood sample used to extract DNA and gene polymorphism implemented used ARMS technique , the results show significant differences between patients and control in age, sex, and no significant differences in residence, the genotype of TNF-α show significant variation between AA and GA genotype in patients and control, A allele in patient was more frequent than control, the present study concluded that variation in TNF-α genotype associated with Rheumatoid arthritis.

**Keywords**: TNF-α, ARMS, Rheumatoid arthritis.

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## INTRODUCTION

Rheumatoid arthritis (RA) is autoimmune disease and chronic inflammatory related with progressive disability, complications in some systems, early death, previous studies show that it was 0.5 to 2% of the world's population (1). the pro-inflammatory cytokines are contributed in RA pathogenesis (2,3). TNF-a and some IL have roles in the pathobiology of RA, researcher improved that IL-1, VEGF and IL-17 also contributed in the process of disease. The level of pro- and anti-inflammatory cytokines were increased in the tissues and serum of patients that indicates the involvement of cytokines in the etiopathology of this disease (4). Tumor necrosis factor (TNF) is a pro-inflammatory cytokine has been role in the pathogenesis of some autoimmune diseases like RA (1,5,6). Rheumatoid arthritis causes by interacted between more than one factors such as genotype, environmental triggers, and chance. Genome wide analyses improved that immune regulatory factors have been the main role in the disease (7) .

McInnes and Schett (8) clarified roles of TNF-a in regulatory of immune system of RA, it causes leukocytes activate , endothelial cells, and synovial fibroblasts, causes production pivotal molecules like cytokines, chemokine's, adhesion molecules, and matrix enzymes, also it have some functions like suppression of regulatory T-cell function, activation of osteoclasts and re sorption of cartilage and bone, it mediates metabolic and dysfunction of cognitive. The Local effects of TNF –α are Increased monocyte activation, cytokine and PG releasing also polymorph nuclear leucocyte priming was elevated and it has role in apoptosis and oxidative burst and synovial fibroblast proliferation, collagen synthesis decrement , , Increased MMP and cytokine release . Systemic effects of TNF –α Acute-phase protein production [10] CVD promotion [11].

## MATERIALS AND METHODS

1- Sample and data collection; about 2 ml of whole blood was collected from patients of Rheumatoid arythritis in Marjan hospital All subjects in this study were taken written consent before participation in this study according to ethical approval of Iraq ministry of health.while control collected from healthy.
2- DNA extraction; DNA was extracted from whole blood using Favor gene extraction kit and concentration and purity were detected using nanodrope.
3- Primers and PCR conditions; primers were used in present study are shown in table (1) (Al-Rayes *et al*., 2011), PCR conditions for ARMS technique were in table (2).
4- Data analysis, PCR products were electrophoresis in 1.5% agarose for 45 min, 70V and 20 mA. The frequency of allele calculated according to hardy-Weinberg law, and the statics analysis implemented using Qi square and odd ration at p value <0.05.

**Table (1) The sequence of TNF-α (G-308A) primers**

| Descriptive | Sequence | Size product |
|---|---|---|
| Sense | 5'-TCT CGG TTT CTT CTC CAT CG-3 | 184 bp |
| Antisense G allele | 5'-ATA GGT TTT GAG GGG CAT GG-3 | |
| Antisense A allele | 5'-AAT AGG TTT TGA GGG GCA TGA-3 | |

**Table (2) PCR conditions for ARMS Technique**

| Subjects | Heat | Time | Cycles |
|---|---|---|---|
| Pre-denaturation | 94 | 5 min | 1 |
| Denaturation | 94 | 15 sec | 10 |
| Annealing | 65 | 50 sec | |
| Extension | 72 | 40 sec | |
| Denaturation | 94 | 20sec | 25 |
| Annealing | 59 | 50 sec | |
| Extension | 72 | 50 sec | |
| Final extension | 72 | 7 min | 1 |

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## RESULTS AND DISCUSSION

The results of present study show that the demographic distribution was shown in table (1) , the age means were 29.441±11.127 for control and 48.22±2.564 for patients according to sex female more than male in patients with significant increment, male percentage was 58.82%, 22.22% in control and patients respectively and female percentage was 38.88%, 77.77% in control and patients respectively, this results deal with international studies that improved that female was more susceptible to the disease than male, this may be because the difference of hormonal activity between male and female also the deference's of behavior and life style (12,13). Disease recorded in urban more than rural, this may because urban life style in Iraq is differs from rural like environmental factors which effect in human health and immunity such as air pollution and stress.

**Table (3) Some Characteristics of Study Groups**

| Categories | C | P | Statics | P-value |
|---|---|---|---|---|
| Age (year) | 29.441±11.127 | 48.22±2.564 | $t$ = 10.8398 | 0.0001* |
| Sex | | | | |
| Male | 58.82% | 22.22% | $X^2$=5.794 | 0.0161* |
| Female | 38.88% | 77.77% | | |
| | | Residence | | |
| Urban | (45.45) | (64.44) | $X^2$=2.528 | 0.1118 |
| Rural | (54.54) | (35.55) | | |

The results of study show DNA extraction from whole blood (figure 1), the TNF –alfa genotype show significant variation between alleles in patient and control gouge, the GG was disappeared in control while it was 5.5% in patients, AA genotype was more frequent in patients (38.88%) than control (5%). GA genotype was more frequent in control (95%) than patients (61.11%), (table, 3) (figure 2). The relative risk analysis show that there is significant variation between patient and control in AA and GA genotype at p-value 0.05 (table 4).

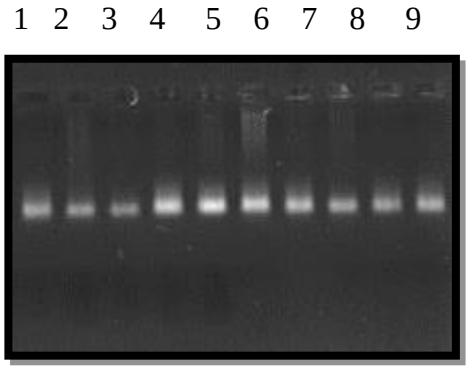


**Figure (1) Electrophoresis pattern of gnomic DNA in study groups, lane 1-5 DNA from patients lane 6-10 DNA from control.**

**Table (4) Distribution of allele frequency and genotype of TNF-α in case-control study.**

| Genotype | Patients% | Control% | $X$ | P- value | Odd ratio | CI 95% |
|---|---|---|---|---|---|---|
| **GG** | 5.55 | 0 | 1.080 | 0.2986 | 0.6000 | 0.0156 - 23.0691 |
| **AA** | 38.88 | 5 | 6.546 | 0.0105* | 12.0909* | 1.3092 - 111.6619 |
| **GA** | 61.11 | 95 | | RG | | |
| **A** | 0.694 | 0.525 | | | | |
| **G** | 0.361 | 0.475 | | | | |

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**Table (5) the Relative Risk of genotyping in study grubs**

| Genotype | Relative risk | CI 95% | P -value |
|---|---|---|---|
| **GG** | | | |
| **AA** | 4.6154 | 0.2031 - 104.8954 | 0.3372 |
| **GA** | 8.4211 | 1.1606 - 61.1014 | 0.0351* |

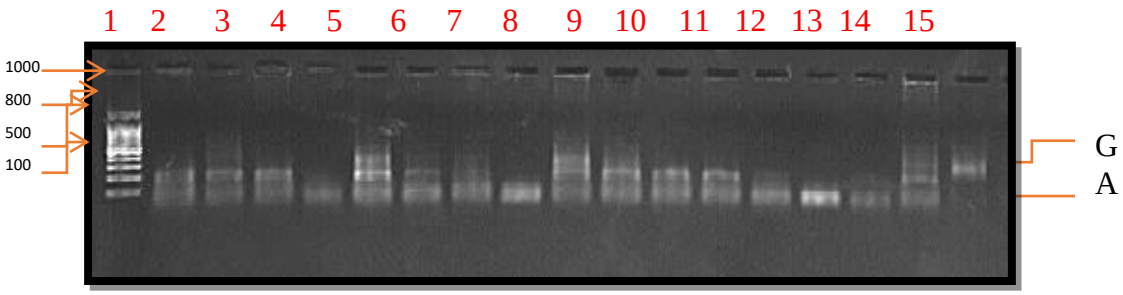


**Figure (2) Electrophoresis pattern of TNF -α genotype in study groups, lane 1 DNA marker (100bp-1kb), lane 2,3,4,6,7,20,11,12,13,14,17 GA genotype, lane 5,9,15 AA genotype, lane 18 GG genotype.**

As a results of the review of literature about TNF-α role in regulatory of immune system in RA it studied in present study using ARMS technique, the studies reported that TNFα −308 polymorphism associated with RA (14,15) . The genetic variation of TNF-alfa in the position −308 lead to two allelic forms in which the presence of guanine (G) defines the common variant and the presence of adenine (A) defines the less common one. TNFα-308A-allele displays increased gene transcription as compared to the common allele G. It has been shown to produce 6–7 fold higher levels of TNF-α transcription (16,17). The results of present study deal with Boechat et al,.(2013) in that the TNF2 allele correlated with the more serious forms disease in pateintes from the Brazilian Amazon but isn't risk of developing RA . Also in Egypt study deal with susceptibility RA with TNF-α-308 G allele and GG homozygous genotype, also it demonstrate A allele with the presence of erosion in the Egyptian patients (19). In meta-analysis implement by Lee et al., (2007) show that the represent a significant risk factor of TNF-alpha -308 A/G polymorphism for RA in Latin Americans, but not in Europeans. In Saudi population study concluded that the TNF-α (−308) and TNF-β (+252) polymorphisms might influence the susceptibility to RA. This present study has prognostic value for future clinical observations of RA patients in Iraqi hospital to used genetic test for estimate patient's response to therapy.. The variation in Iraqi population may be because the life style of individuals or elevated oxidative stress which causes accumulation of ROS, this causes different variation in DNA sequence, gene expression and its regulation, also studies which deal with Iraqi population improve that Iraqi patients suffered from contributed more than one factor which causes disease (20). The environmental pollution have been main role in incidence disease in Iraqi patients like heavy metals (21, 22).

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