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INVESTIGATING THE INFLUENCE OF NF-KB SNP ON RHEUMATOID ARTHRITIS IN IRAQI PATIENTS

Ahmed Shaker Dewan*[1] and Syoof Khoman Alwan[2]

[1,2]University of Al-Qadisiyah, College of Science, Department of Biology, Iraq.

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***Corresponding Author: Ahmed Shaker Dewan**

University of Al-Qadisiyah, College of Science, Department of Biology, Iraq.

ABSTRACT

This study aims to investigate the impact of single nucleotide polymorphisms (SNPs) (rs4648068) of the NF-κB gene on rheumatoid arthritis patients in Iraq, providing a basis for future research and potential therapeutic interventions. Blood samples were collected and divided into two groups: the first group consisted of 50 samples from patients with rheumatoid arthritis, the second group consisted of 50 samples from healthy individuals without the disease, and the last group represented a control group.

KEYWORDS: *NF-κB, RA, SNP, rs 4648068, promoter, polymorphism.*

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease that primarily affects the joints, leading to chronic inflammation, cartilage damage, and bone erosion.[1][6] The pathogenesis of RA involves a complex interplay of genetic, environmental, and immunological factors.[6][7]

Genetic factors contribute significantly to the risk of developing RA. Specific alleles, such as those in the major histocompatibility complex (HLA-DR4 and HLA-DR1), are associated with increased susceptibility.[6] However, the onset of the disease typically requires interaction with environmental factors.[6] Dysregulation of the immune system, particularly TH17 cells and cytokines such as IL-17, IL-6, and TNF-α, contributes to the development of rheumatoid arthritis.[7]

The nuclear factor-kappa B (NF-κB) signaling pathway is a critical regulator of inflammation and immune responses.[3][4] Dysregulation of the NF-κB pathway has been implicated in the pathogenesis of RA.[4][5] NF-κB regulates the expression of numerous genes involved in inflammation, including cytokines, chemokines, and adhesion molecules[4] Activated NF-κB is observed in RA synovium in early and late stages of joint inflammation, and initiation of inflammation is triggered by NF-κB activation in both T cells and antigen-presenting cells.[4]

Single nucleotide polymorphisms (SNPs) within the NF-κB pathway may influence the activity of the pathway and, consequently, affect the risk and severity of RA.[3][8] Identifying specific NF-κB SNPs associated with RA in different populations may provide valuable insights into the genetic architecture of the disease and contribute to personalized medicine approaches.[3]

The Iraqi population, with its unique genetic background, may harbor specific NF-κB SNPs that contribute to RA susceptibility.[1][9] Therefore, this study aims to investigate the association between NF-κB SNPs and RA in a sample of Iraqi patients, providing a foundation for future research and potential therapeutic interventions.[2][10]

METHODS

Patients and Study Design

This study was conducted in two groups, as shown in Figure-1. The first group included 50 patients with rheumatoid arthritis (9 males and 41 females). The second group included 50 healthy patients (without rheumatoid arthritis) as a control group (28 males and 22 females). 5 ml blood samples were collected from patients who visited Al-Diwaniyah Teaching Hospital, Marjan Teaching Hospital, private clinics, and outpatient clinics. They were diagnosed using specific clinical and immunological tests.

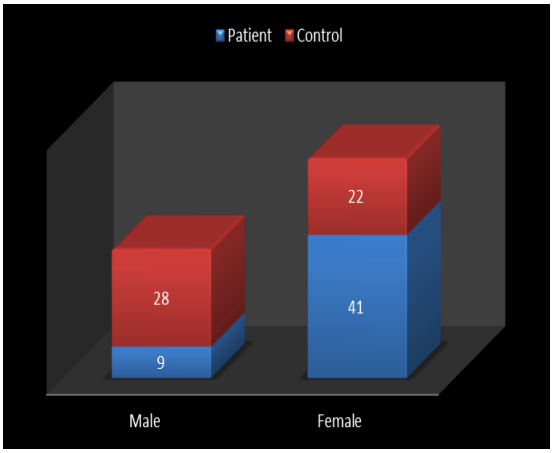

Figure 1: Distribution of the group of RA patients and the control group for study samples by gender.

Genetic of NF-κB / rs 4648068 polymorphism
Blood Genomic DNA Extraction
Using a DNA extraction kit blood samples and blood genomic DNA was extracted from the gSYAN(frozen blood protocol) Geneaid. According to the instructions of the company the USA.

Estimation of DNA in Blood
Genomic DNA was tested for blood extracted using a nanoscale Spectrophotometry (THERMO.USA), measures the The concentration DNA (ng/µl), absorbance (260/280 nm) reading at and DNA checked are purity.C- Preparing of the primer's suspension.[11]

Preparation of Suspended Primer
The Tetra-ARMS-PCR primers for detecting the gene polymorphism were designed in this study using the NCBI-SNP database in conjunction with the ARMS-PCR Primer Design Server (https://snp.biotech.edu.lk/arms.php). The primers were synthesized and supplied by Scientific Researcher Co. Ltd., Iraq, as detailed in the following tables.

Table 1: The Tetra-ARMS-PCR Primers for NFKB1 single nucleotide polymorphism (SNP) rs4648068 gene polymorphisms with their sequence and amplicon size.

T-ARMS-PCR Primer	Sequence (5'-3')	Product size
Forward inner primer (G allele):	GCCTAACAAGCTAATTGTTAGAGATTCAAG	**388bp**
Reverse inner primer (A allele)	CACCAATATCTTGGTGAAATAATCCTTAAT	**445bp**
Forward outer primer	TTATCTTGTGCCCTCTTTGAAGAAGTA	**773bp**
Reverse outer primer	CCACAATCATAGTTGAGTGTTCCTACTT	

Statistical Analysis
Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS version 29.0) https://spss.softonic-ar.com/. The relevant P-value as an indicator of significance was determined, and $P \leq 0.05$ was considered statistically significant in all tests.as P 0.05 in all tests.

RESULTS

Detection of NF-κB / rs 4648068 A > G SNP
The distribution of the NF-κB/rs4648068 SNP was detected by ARMS-PCR. There are three genotypes at this locus: the product size of the G allele: 388 base pairs, and the product size of the A allele: 445 base pairs. Genotype analysis revealed three genotypes (GA, GG, and AA). When genotype distribution of the NF-κB/rs4648068 SNP across the study groups, the GA genotype was more common in patients (0.25) and less common in the control groups (0.13). On the other hand, the AA genotype was more prevalent in the control group (0.33) compared to the inpatient group (0.17). The GG genotype was less common in both the in patient group (0.08) and the control group (0.04). There was a statistically significant increase in the incidence of AA and GA and a decrease in the incidence of GG genotypes in both the inpatient and control groups ($P < 0.05$).

Table 2: Distribution frequencies of NF-κB/rs4648068 SNP alleles among the study group (control group and patients).

SNP	Allele	Frequency	Controls	Patients	P value	OR (95% CI)
Rs 4648068	**A**	138 (0.69)	79(0.79)	59(0.59)	< 0.01 (HS)	0.38 0.20 to 0.71
	G	62 (0.31)	21(0.21)	41 (0.41)	< 0.01 (HS)	2.61 1.40 to 4.88

DISCUSSION

Polymerase chain reaction (PCR) using the quadruple amplification mutation system was used to detect the NF-κB / rs4648068 A > G SNP polymorphism in Iraqis. The A allele of the NF-κB / rs4648068 A > G SNP was associated with a moderately reduced risk of rheumatoid arthritis (OR = 0.38, 95% CI = 0.20 to 0.71, p < 0.01). The G allele of the NF-κB / rs4648068 A > G SNP was associated with a moderately increased risk of rheumatoid arthritis (OR =2.61,95% CI = 1.40 to 4.88, p < 0.01). We also analyzed different genotypes in patients and healthy controls; the proportion of the A allele in

patients was (59%), compared to the control group (79%), while the proportion of the G allele in patients was (41%), compared to the control group (21%).

Specifically, the A allele was observed at a lower frequency among RA patients than controls, indicating a moderately protective effect against RA. Conversely, the G allele was more prevalent among patients, suggesting a moderate increase in risk. These findings are consistent with the role of the NF-κB signaling pathway in chronic inflammation and autoimmune pathogenesis. NF-κB1 is a transcription factor that regulates inflammatory gene expression, immune cell activation, and cytokine production—all of which are implicated in RA pathophysiology.[12] Recent evidence has shown that polymorphisms within the NF-κB1 gene can affect the behavior of **fibroblast-like synoviocytes (FLS)**, which are key effector cells in RA. Activated FLS exhibit enhanced resistance to apoptosis and increased secretion of proinflammatory mediators such as IL-6 and TNF-α, processes largely regulated by NF-κB activation.[13] Variants such as rs4648068 may influence NF-κB1 expression or function, thereby modifying the inflammatory threshold in genetically susceptible individuals. Interestingly, a study in Egyptian patients with Behçet's syndrome reported that the **GG genotype** of rs4648068 was associated with a reduced incidence of arthritis, suggesting a context-dependent effect of this polymorphism on immune-mediated diseases.[14] Moreover, other research has proposed that interactions between **NFKB1**, **IL1β**, **IL18**, and **IFNG** genes may collectively contribute to the **severity** of RA rather than its onset, indicating that SNPs in NF-κB1 could play a more prominent role in disease progression.[15] Although the observed associations in our study are moderate, they underscore the potential value of rs4648068 as a genetic biomarker in RA risk assessment within the Iraqi population. Nevertheless, larger and ethnically diverse cohorts are needed to validate these findings. Furthermore, functional studies are essential to determine whether this SNP affects gene transcription, mRNA stability, or alternative splicing of NF-κB1 in immune-relevant tissues.

CONCLUSION

In summary, this study highlights the potential role of NF-κB genetic polymorphisms, particularly rs4648068 (A>G), in modulating susceptibility to rheumatoid arthritis (RA) within the Iraqi population. The observed association between the G allele and increased RA risk, along with the protective tendency of the A allele, suggests a functional relevance of this SNP in the pathogenesis of RA. These findings reinforce the importance of the NF-κB signaling pathway in autoimmune inflammation and underscore the value of population-specific genetic studies in uncovering novel risk variants. Future investigations with larger cohorts and functional analyses are warranted to validate these associations and explore their utility in personalized medicine and targeted therapeutic strategies for RA.

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