Genetics Research

Role of genetic variation in Toll like receptor-5 with rheumatoid arthritis pathogenicity in Iraqi patient

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Abstract

Background: Rheumatoid Arthritis (RA) is a persistent inflammatory condition characterized by the immune system's improper attack of bodily tissues. Rheumatoid arthritis is a chronic autoimmune disorder that impacts the synovial membrane of joints, leading to an inflammatory response characterized by painful swelling. Over time, this condition can progress to the erosion of bone tissue and the development of joint deformities. Aim: characterized the primary objective of the present study was to evaluate the impact of certain variations in the TLR5 gene (namely, rs2072493 and rs45508499) on the severity of Rheumatoid Arthritis among patients from Iraq. Materials and methods: Approximately similar samples from apparently healthy individuals are enrolled as control. The patients attended from April to June 2022 at the Baghdad teaching hospital / medical city /Baghdad / Iraq. It was based on a clinical examination . The single nucleotide polymorphisms (rs2072493 T/A, C, and rs45508499A/G) are examined using the Tetra ARAMS technique. All (SNPs) from the control person are analyzed in the Chi-square test. Results: The analysis revealed a significant association between the rs45508499 polymorphism and the trait of interest. However, no significant association was observed for the rs2072493 polymorphism. Conclusion: The current investigation showed that the presence of TLR5 polymorphism at rs4550899 was associated with RA but   rs2072493 was not associated with any effect on the susceptibility to Rheumatoid arthritis .

Keyword : Toll like receptor-5 gene , rs2072493, rs45508499 ,Tetra arms PCR ,Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease defined by inflammation of the articular tissue[1], which results in joint deformities and impairment in women aged (30-50) . Rheumatoid arthritis (RA), which affects about 1% of adult humans globally and is more prevalent in women (3:1 female to male ratio), may affect at any age[2]. Late childbearing years are the normal age of onset for women; the sixth to eighth decade is when RA tends to develop more frequently in males [3]. Rheumatoid arthritis (RA) is a type of arthritic condition characterized by joint inflammation, discomfort, and swelling. Typically, the condition is characterized by chronicity and has the potential

to result in enduring joint injury or deformity [4]. Toll-like receptors (TLRs) represent a prominent category of pattern-recognition receptors within the innate immune system. These receptors play a crucial role in recognizing and detecting pathogen-associated molecular patterns (PAMPs) derived from many infectious diseases [5]. All Toll-like receptors are classified as type 1 transmembrane proteins. In the human population, a total of ten Toll-like receptor (TLR) subtypes have been identified and classified. The cell surface contains TLRs 1, 2, 4, 5, 6, and 10, while the endosomal membrane has TLRs 3, 7, 8, and 9. These Toll-like receptors primarily play a role in the innate immune system and regulate pro-inflammatory pathways [6]. Toll-like receptors (TLRs) are capable of triggering immunological and inflammatory responses by activating inflammatory cells in the presence of invading microorganisms. The relationship between the activation of Toll-like receptors (TLRs) and rheumatoid arthritis has been established in the context of flagellin, a bacterial

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structural protein (7).Recent research has provided evidence of increased TLR5 receptor expression in cartilage lesions associated with osteoarthritis. This finding suggests that aberrant TLR signaling may play a role in the development of rheumatological disorders beyond just rheumatoid arthritis. Furthermore, research on the TLR pathway has yielded novel insights into the etiology of rheumatoid arthritis.

Methods

Subject

In this study, 100 subjects participated as (n=50)patients suffering from Rheumatoid arthritis (RA), and 50 appeared to be healthy controls. From April to June 2022 at the Baghdad teaching hospital / medical city /Baghdad / Iraq and (n=50) appeared to be healthy controls with no family history of Rheumatoid arthritis. It is based on a clinical examination. All patients' Blood samples collection in this research study are extended to A period of(1 march-2023 to30 May-2023) Blood samples are obtained from each participant using venipuncture, with a volume of 10ml collected per sample. Each sample is then divided into two portions: one portion consisting of 3ml of blood is placed in an EDTA tube for the purpose of conducting a Hematological test. The EDTA tube is designed to accommodate a volume of 4 mL of blood, which is then utilized for the purpose of DNA extraction. The gel tube consists of 3ml of blood that has been separated using centrifugation. Subsequently, the resulting serum is divided into three distinct tubes, each comprising 1ml. These tubes are allocated for conducting tests related to C-reactive protein (CRP), rheumatoid factor (RF), and anti-cyclic citrullinated peptide (anti-CCP) antibodies.

SNP selection

Two SNPs were selected in the TLR5 gene region (Rs 2072493 and Rs45508499). SNPs were (http://www.ncbi.nlm.nih.gov/SNP, BUILD130). Peripheral blood samples were collected Individuals who were chosen for the study and had uncertain heterozygosity and minor allele frequencies lower than 5% were eliminated from the analysis. The DNA Extraction kit (Trans Gene Company, China) was utilized to extract genomic DNA from samples collected in EDTA tubes. The integrity of the sample was assessed using agarose gel electrophoresis, while the purity and concentration were determined by Nanodrop analysis. The DNA that has been extracted is stored at a temperature of -20°C until it is ready to be utilized. The TLR5 genotype was determined using the Tetra allele refractory mutation system-polymerase chain reaction (TETRA-ARMS). The reaction was conducted in a volume of 20μL, comprising 4μL of DNA, 1μL of outer primers, 2μL of inner primers, and 10μL of distilled water.

Genotyping of SNPs

The forward primer, 5`ACATTGGTGTGATTAAGCCCA 3` , and the reverse primer, 5`CAACAGGCTGA-CAGTTCTTTCTC 3`, were arranged in the appropriate sequence and proportion (1:2, Forward: Reverse). The reverse primer sequence is 5`TGTGAATGTGAACTTAGCACTTT-TATAAG3`, while the inner primers include the sequence 5`GGAACTTTGTGACTGTGAGGATG3`.

Polymerase chain reaction (PCR) cycles

the polymerase chain reaction (PCR) procedure was executed, comprising an initial denaturation phase with a duration of 5 minutes at a temperature of 95 °C, followed by 30 cycles, each lasting 40 seconds, and concluding with a final extension step of 5 minutes at a temperature of 72 °C.

Table 1: information of SNPs in TLR5

GENE TLR5	Ref *Homo sapiens*	Highest population MAF	Chro	SNP site	Functional Consequence	Clinical significant
NC 000001.11:223111857_	Rs2072493T>A,C	0.36	one	Exon5	missense variant	risk factor
	Rs45508499 G>A	0.50	one	down-'3 stream sequence	3primeUTRvariant	NON RE- - GESTERS

Data analysis

The Statistical Analysis System- SAS (2012) program was employed to modify multiple study parameter elements. In this study, the least significant difference (LSD) test and the chi-square test were used to compare percentages and means in a statistically significant way. The odd ratio was also employed to identify risk factors (SAS, 2012).

Results

Distribution of Participants according to Gender

According to the current study, there were more female patients (40.8%) than male patients (27.6%). Additionally, the proportion of females among patients was higher than in the control group of the healthy individuals (Figure:1).

3.2 The association of TLR5 (Rs 2072493 and Rs45508499)

with Rhematoid arthritis patients .

TLR5 rs2072493 , the first position has three genotypes (TT,TC,CC), with TC being non significantly associated with RA patients where it was accounted for (50(100%)vs (46(90%).with P value =0.96). The TC and CC was nonsignificant in the influence between rs2072493 polymorphism and RA.

Table 2: Stratification analysis of TLR5 (rs2072493 T>C) Genotypes and alleles in RA patients and persons healthy

Genotype Frequency (%) rs2072493						
Genotype	Control n=50	Patients n=50	P-value	Chi-square	Odds Ratio	Cl 95%
TT	(0%) 0	(0%) 0	----	----	1.0	----
TC	(100%) 50	(92%) 46	NS 0.9673	----	1.0860	to 14.7593 0.0008
CC	(0%) 0	(8%) 4	NS 0.3784	----	0.1111	to 19.42 1.315
Chi-square	** 50.0	** 36.28				
p Value	0.0001>	0.0001>				
(%) Allele frequency						
Allele	n=100	n=100	P-value	Chi-square	Odds Ratio	Cl 95%
T	(50) 0.5	(46) 0.46	NS 0.5713	0.3205	0.8519	to 1.493 0.4835
C	(50) 0.5	(54) 0.54				

But in TLR5 (Rs45508499), the lengths of a fragment of specific amplicons as (A allele) was (189 bp), and the G allele was (255bp). There are two specific heterozygosity amplicons; moreover, amplicons (non indicative) result from the primer pairs (395 bp for the Two outer primers). As illustrated in table 2, AA genotype decreased in patients (40%), as compared to (16%) in healthy controls with nonsignificant . There was significant difference between those groups with the frequency of AG and GG genotype (52%) vs. (66%) with (P=0.01) for AG genotype and (8%) vs (18%) with (p=0.01) for GG genotype.

Table 3: Stratification analysis of TLR5 (rs45508499A>G) Genotypes and alleles in RA patients and persons healthy.

Genotype Frequency (%) rs45508499						
Genotype	Control n=50	Patients n=50	P-value	Chi-square	Odds Ratio	Cl 95%
AA	(40%) 20	(16%) 8	----	----	1.0	----
AG	(52%) 26	(66%) 33	* 0.0169	5.705	3.173	to 8.477 1.159
GG	(8%) 4	(18%) 9	* 0.0139	6.047	5.625	to 19.42 1.315
Chi-square	NS 1.258	NS 5.124				
p Value	0.5330	0.0771				
(%) Allele frequency						
Allele	n=100	n=100	P-value	Chi-square	Odds Ratio	Cl 95%
A	(66) 0.66	(49) 0.49	* 0.0150	5.913	2.020	to 3.550 1.130
G	(34) 0.34	(51) 0.51				

Discussion

Rheumatoid arthritis (RA) is a persistent inflammatory condition that affects the joints and has a systemic impact(8) . It primarily affects women and is characterized by the development of autoantibodies targeting immunoglobulin G (IgG), known as rheumatoid factor, as well as citrullinated proteins, referred to as anti-citrullinated protein antibodies (9). The investigation of gender disparities in rheumatoid arthritis (RA) has primarily focused on the role of sex hormones. Estrogens exhibit a dual effect on the immune system, characterized by the downregulation of inflammatory pathways and the upregulation of immunoglobulin synthesis (10). The diverse impacts of estrogens on immune function are influenced by both the levels of estrogen present and the specific distribution and subtype of estrogen receptors found in immune cells (11). There appears to be a higher level of TLR 5 expression in the immune cells of females in comparison to males, which results in increased cytokine production by female immune cells. This phenomenon is influenced by the expression of sex chromosomes (12). Recent epidemiological research has established smoking as a significant risk factor for rheumatoid arthritis (RA) (13). Several research has indicated that smoking has a greater impact on the chance of developing rheumatoid arthritis (RA) in males compared to women (14). However, several reports suggest smoking increases the risk of developing RA in women (15). A recent study was done to perform the inaugural meta-analysis on the relevance of smoking as a potential risk factor for the development of rheumatoid arthritis (RA). The findings of this study indicate that smoking does definitely pose a danger to the development of RA, particularly in RF-positive men and individuals who engage in heavy smoking. The incidence of rheumatoid arthritis (RA) was shown to be nearly twice as elevated in individuals who smoke compared to those who do not engage in smoking. The risk of smoking-related health issues for women who smoke was shown to be roughly 1.3 times greater compared to those who do not smoke. Despite the lack of conclusive evidence from earlier research about the correlation between smoking and the onset of rheumatoid arthritis (RA) in women, Sugiyama et al. presented quantitative findings that establish smoking as a substantial risk factor for the development of RA in women (16). The user's text is already academic and does not require any rewriting .TLRs may act as receptors for damage-associated molecular patterns. Although TLR5 was not considered as important damage-associated molecule receptor (17), TLR5 is located on the chromosomal position 1q41-q42 and the locus was associated with systemic lupus erythematosus, RA , and other autoimmune diseases (18) . Genetic variations of the TLR5 gene have been studied in the aspects of andrology and urology. TLR5 single nucleotide polymorphisms (SNPs) including a missense SNP rs2072493 and 3-prime-UTR SNPs Rs45508499 were tested in the prostate cancer population and RA , however, they were not significant (19). rs2072493 and rs45508499 was mentioned in two previous studies. It was tested between the cases of posterior longitudinal ligament of the spine and controls, however, not significant along with other TLR5 polymorphisms .(20) And rs2072493 was tested in rheumatoid arthritis patients and in anti-tumour necrosis factor (TNF) treatment of RA, however none of them were significantly associated (21). Our study result may show some differences compared to previous studies, because the genotypes and allele frequencies were significantly associated with BPH development in rs45508499.

Conclusions

In summary, the findings of this investigation indicate the presence of genotype AG and GG of TLR5 rs45508499 in Iraqi individuals diagnosed with rheumatoid arthritis (RA), so establishing a potential association between TLR5 single nucleotide polymorphisms (SNPs) and the severity of RA in the Iraqi population. Additional research is required to examine the biological consequences of the mutations under investigation on various cytokine production and TLR5 signaling pathways. Furthermore, it is necessary to explore the impact of TLR5 polymorphisms on the course of rheumatoid arthritis (RA) in diverse ethnic populations.

Conflict of interest:

The authors indicate that they have no problems of interest.

Authors contribution:

Ali Hussein Alwan and Saad L Hamed designed the experiments and performed experiments . Aliaa khauon conducted the sampling, executed the experiments, composed the basic draught of the manuscript, conducted phenotypic research, analyzed the data, and authored the manuscript. The manuscript underwent a thorough review and revision process, with input and edits provided by all authors.

Ethical statement:

The work has been reviewed and approved by the Ethics Committee of the College of Science at Mustansiriyah University on February 1, 2022, under reference number BCSMU/1221/0005M. The research adheres to the highest ethical standards for human subject research, including informed consent, confidentiality, and data security. All participants provided informed consent for their participation, and their privacy and well-being have been prioritized throughout the research process.

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