Investigating the Association Between VCAM-1 Gene Polymorphism and Rheumatoid Arthritis Susceptibility in a Sample of Iraqi Patients

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Abstract: Vascular cell adhesion molecule-1 (VCAM-1) plays a significant role in the mediation of leukocyte adhesion and migration, and its role has been implicated in the pathogenesis of rheumatoid arthritis (RA). Even though there are genetic variations in the VCAM-1 gene, there is insufficient data regarding the role of the rs3170794 polymorphism, especially among Iraqi populations. A study investigated the VCAM-1 rs3170794 polymorphism and RA risk in Iraqi patients. The current study was conducted on 30 RA patients and 20 healthy controls were enrolled. Genomic DNA was extracted from peripheral blood, and the genotype of the VCAM-1 rs3170794 polymorphism was identified using conventional PCR and sequencing. After that, genotype and allele frequencies were calculated and compared to those expected under the Hardy-Weinberg principle. The relationship between the VCAM-1 rs3170794 polymorphism and RA risk was calculated using odds ratios with 95% confidence intervals and Fisher's exact test. The results showed that there are three genotypes: TT, CT, and CC. There are also two alleles: T and C. Among these genotypes, TT is the most dominant genotype. It is seen in 90% of the patients and 95% of the control group. What is also interesting is that the CC genotype is only found in RA patients (6.67%) but not in the control group. Although the risk of RA is seen with the CC genotype (OR 3.60) and C allele (OR 3.55), these are not statistically significant. All the genotypes were in Hardy-Weinberg equilibrium. In conclusion, the polymorphism of VCAM-1 rs3170794 did not demonstrate a statistically significant association with susceptibility to RA. Nevertheless, the increased prevalence of the CC genotype and C allele in patients with the condition indicates a possible association, which should be further explored. This should be done with a larger sample size, including functional studies to understand the impact.

1 INTRODUCTION

Cell adhesion molecules (CAMs) play an important role in many biological processes. They are involved in cell adhesion and locomotion, differentiation, and inflammatory and immune responses. In addition, cell adhesion molecules are also involved in the pathophysiology of inflammatory and immune disorders. Their role in inflammatory and immune disorders is evident in rheumatoid arthritis (RA) and other disease processes [1], [2]. Vascular cell adhesion molecule-1 (VCAM-1) is a glycoprotein on the cell membrane that is part of the immunoglobulin superfamily. VCAM-1 plays an important role in the pathophysiology of rheumatoid arthritis. Its major role is the mediation of leukocyte adhesion and transmigration through the endothelium of the blood vessels into the inflamed synovial tissue [3]. VCAM-1 is mainly expressed on the surface of endothelial cells that line activated vessels. It binds with integrin α4β1, also called VLA-4. Integrin α4β1 is expressed on the surface of various leukocytes, including T cells, B cells, monocytes, eosinophils, and basophils. But the major interaction is between integrin α4β1 and VCAM-1; this interaction is responsible for the adhesion of leukocytes with the endothelium of blood

vessels, thus facilitating their transmigration into the inflamed synovial tissue, an important event in the pathophysiology of rheumatoid arthritis [4]. In the context of rheumatoid arthritis, the role of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) is critical, and this occurs through the stimulation of vascular endothelial cells. This, in turn, leads to the upregulation of adhesion molecules such as VCAM-1 and ICAM-1. This leads to the adhesion of leukocytes to the endothelium and further facilitates their migration into the synovial membrane, thereby resulting in the persistence of inflammation and destruction of joint structures [5]-[7]. Studies have shown that serum vascular cell adhesion molecule-1 (VCAM-1) levels are significantly higher among rheumatoid arthritis (RA) patients than in healthy individuals. Additionally, there is a positive correlation between the degree of elevation of VCAM-1 and the degree of disease severity. Interestingly, the concentration of VCAM-1 decreases progressively with the disease activity in response to certain biological targeted therapies, like tumor necrosis factor-alpha (TNF-α) inhibitors. This corresponds to VCAM-1's value as a potential biomarker for monitoring disease activity and response to treatment in RA [8], [9].

Although the immunological processes in rheumatoid arthritis are being studied and better understood, the specific contribution of some adhesion molecules like the vascular cell adhesion molecule-1 (VCAM-1) in perpetuating synovial inflammation and worsening tissue destruction is still unclear. Recent studies also show that increased VCAM-1 expression may be associated with an increased risk of some vascular complications, including atherosclerosis, which adds another layer of complexity to rheumatoid arthritis [3], [10]. Focusing on the molecular details, the VCAM-1 gene is located on the short arm of human chromosome 1 at 21p21.2, and spans nine exons. There is a need for further molecular study of this gene, as the SNPs (single nucleotide polymorphisms) that lie in the gene itself and/or in the promoter region can alter the activity, control, and expression of the resultant protein [11]. Therefore, polymorphisms in the VCAM-1 gene may be relevant to the pathogenesis of inflammatory diseases, such as rheumatoid arthritis (RA) [4].

There have been some specific genetic variations, which have been found to be of great functional significance, located in the VCAM-1 gene, especially in the region of the promoter, which is known to play an important role in the regulation of the expression levels of the gene [12]. For example, the VCAM-1-related pathology has been linked to the rs3783605 single nucleotide variation, which might be attributed to the location of the SNP in the region of the ETS2 transcription factor-binding site of the VCAM-1 promoter region [13]. In addition, there have been some specific studies that have revealed the significance of the VCAM-1 1238 G>C polymorphism in the context of rheumatoid arthritis (RA) pathogenesis. The VCAM-1 G>C genotype has been found to be of great significance, especially in African American populations, where it is found to be significantly higher (27%) than in the Mexican population. This disparity suggests that this polymorphism could serve as a valuable genetic marker for RA susceptibility, exhibiting population-specific relevance [14]. Other research has investigated the association of four VCAM-1 SNPs: rs3176860, rs2392221, rs3917010, and rs3176879, in association with obesity and inflammatory markers. The findings showed that genetic variation in VCAM-1 might be linked to obesity and inflammatory markers in the Korean population [15]. The VCAM-1 and ICAM-1 gene polymorphisms have been known to be linked to the expression of these molecules, which might affect their inherent role in the immune response in various diseases [16]. Studies have been done on the VCAM-1 gene polymorphism (rs3170794) as it relates to different inflammatory conditions. There are no studies that have shown the connection between this polymorphism and susceptibility to rheumatoid arthritis specifically in the Iraqi population. Therefore, this study is being conducted to evaluate if rs3170794 could be a risk factor for RA in that ethnic group.

2 MATERIALS AND METHODS

2.1 Study Design

The case-control study was conducted between November 2023 and March 2024, recruiting 30 Iraqi patients between 25 and 70 years old, selected from the Rheumatology Unit of Baghdad Teaching Hospital and Baquba General Hospital. In addition, there was a control group of 20 healthy people, consisting of 16 women and 4 men. All of the recruited patients were confirmed to have rheumatoid arthritis, fulfilling the 2010 ACR/EULAR criteria for rheumatoid arthritis [17]. For genetic analysis, we used 3 mL of venous blood from each individual, collected using an EDTA tube to obtain DNA and analyze polymorphisms within the selected genetic region.

2.2 Extraction of Genomic DNA

Genomic DNA was isolated from EDTA blood samples using the ReliaPrep™ Blood gDNA Miniprep System kit (Promega, USA). The extracted DNA exhibited a purity range of 1.7–2.0 and concentrations between 50 and 100 ng/mL. Subsequently, the DNA was subjected to polymerase chain reaction (PCR) amplification. Genotypes were characterized using the sequence-specific primer (SSP) method. Sequence-specific primers for amplification of the VCAM-1 rs3170794 single nucleotide polymorphism (SNP) were designed using the PRIMER3PLUS program. The forward and reverse primers were designed to produce an 867 bp PCR product. The primer sequences and amplification conditions are presented in Table 1.

2.3 Detection of the VCAM-1 polymorphism (rs3170794) using conventional polymerase chain reaction (PCR).

Primers were obtained as lyophilized products from Bioneer (Korea) at various picomolar quantities. In accordance with the manufacturer's instructions, primers were reconstituted in nuclease-free water to obtain a stock solution with a final concentration of 100 pmol/µl. Subsequently, 10 µl of the stock solution were diluted with 90 µl of nuclease-free water to prepare a working solution at a final concentration of 10 pmol/µl for use in conventional PCR.

Each 25 µl Polymerase Chain Reaction (PCR) mixture comprised 5 µl of PCR pre-mix (Bioneer), 1.5 µl of forward primer, 1.5 µl of reverse primer, 3 µl of DNA template, and 14 µl of deionized water (D.W.). PCR amplification was performed using the following thermal cycling conditions: an initial denaturation step at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. As a last step of the PCR process, an extension phase was performed at 72 degrees Celsius for five minutes. Each of the PCR reactions was resolved via gel electrophoresis in a 1.5% agarose gel for 90 minutes at 90 volts. The gel was stained with ethidium bromide and viewed on an ultraviolet transilluminator using a 350 nm wavelength light, as compared with a DNA ladder from Intron Company (South Korea).

2.4 PCR Amplification Products

Using an ABI 3730xl DNA Analyzer (Macrogen Corporation, Seoul, Republic of Korea), PCR amplicons were sequenced. The raw sequence files were sent via email to the researchers, who then prepared the sequence data for further analysis using the Geneious Prime software, version 2020.1 (Biomatters Ltd., Auckland, New Zealand; available at www.geneious.com) [18].

2.5 Statistical Analysis

The frequency of genotypes for the VCAM-1 rs3170794 polymorphism was calculated as a percentage. Two-tailed Fisher's exact probability test, denoted as P-value, was used to check for significant differences in the distribution of genotypes between patients and controls. Moreover, the etiologic fraction (EF) and preventive fraction (PF) were also computed. The association of each genotype with the disease was also checked by calculating the odds ratio with 95% CI. All calculations were done using the WINPEPI software for epidemiologists, version 11.63 [19].

2.6 Ethical Approval

The study protocol was approved by the Ethics Committee of the University of Diyala and the Council of the College of Education for Pure Sciences. All procedures involving human participants were conducted in accordance with institutional ethical standards and the principles of the Declaration of Helsinki.

RESULTS

PCR amplification of the VCAM-1 gene fragment containing the rs3170794 polymorphism consistently produced a single amplicon of approximately 867 bp, as shown in Figure 1.

Table 1: Primers used to amplify gene VCAM-1(rs3170794).

Primer	Sequence of primer	Product Size	Annealing temperature	Design
F-VCAM-1	GGCTATGTGTGTGCAAGGCT	867	60	Current study
R-*VCAM-1*	CCTTCAAGGGGAAACCCAGG			

Note: F, forward primer; R, reverse primer.

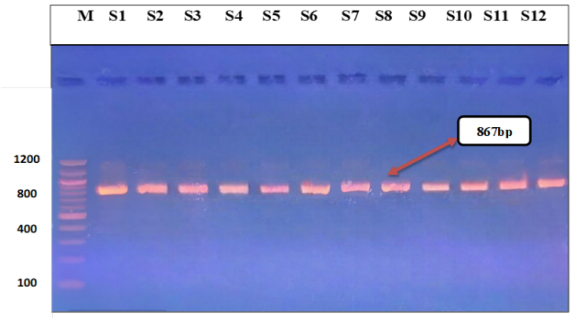

Figure 1: Agarose gel electrophoresis of PCR-amplified VCAM-1 (rs3170794) products.

Figure 1 shows the results of agarose gel electrophoresis of PCR-amplified VCAM-1 (rs3170794) products. Ethidium bromide staining produced a distinct band of approximately 867 bp (as seen with UV illumination). The electrophoresis was performed on a 1% agarose gel at 90 V for 90 minutes. Lane M denotes the 100-1200 bp DNA ladder, while Lanes S1-S10 represent individual samples obtained from patients with rheumatoid arthritis.

Analysis of the single nucleotide polymorphism (SNP) rs3170794 revealed the presence of three distinct genotypes (TT, CT, and CC) and two corresponding alleles (T and C), as visually represented in Figure 2. The genotype and allele frequencies for the VCAM-1 rs3170794 polymorphism in both the control and patient cohorts are presented in Table 2. All observed genotype frequencies within both study groups were found to be in Hardy-Weinberg Equilibrium (HWE). Furthermore, statistical analysis indicated significant differences in the genotype and allele frequencies of VCAM-1 rs3170794 in the rheumatoid arthritis patient group ($p < 0.001$) and no significant differences in the genotype and allele frequencies of the control group ($p > 0.9087$).

Our study analysis (Table 3) indicated a low frequency of the mutant homozygous (CC) genotype for the polymorphism under investigation. The homozygous CC genotype did not appear in any convincing way, to bear much relation to the likelihood of rheumatoid arthritis, when the patients were set beside the control series ($X^2 = 0.333$, OR = 3.60, 95% CI 0.18 to 73.61). A slight increase in the frequency of the CC genotype was observed in the patient group, roughly (6.67) percent, while in the controls none at all was recorded.

Similarly, the heterozygous CT form did not show relation to the likelihood of rheumatoid arthritis. The distribution differed only slightly between the two sets ($X^2 = 0.755$, OR = 0.66, 95% CI 0.02 to 26.84). whereas the control group showed a slightly higher frequency, around 5 percent whereas the patients showed only about (3.33) percent.

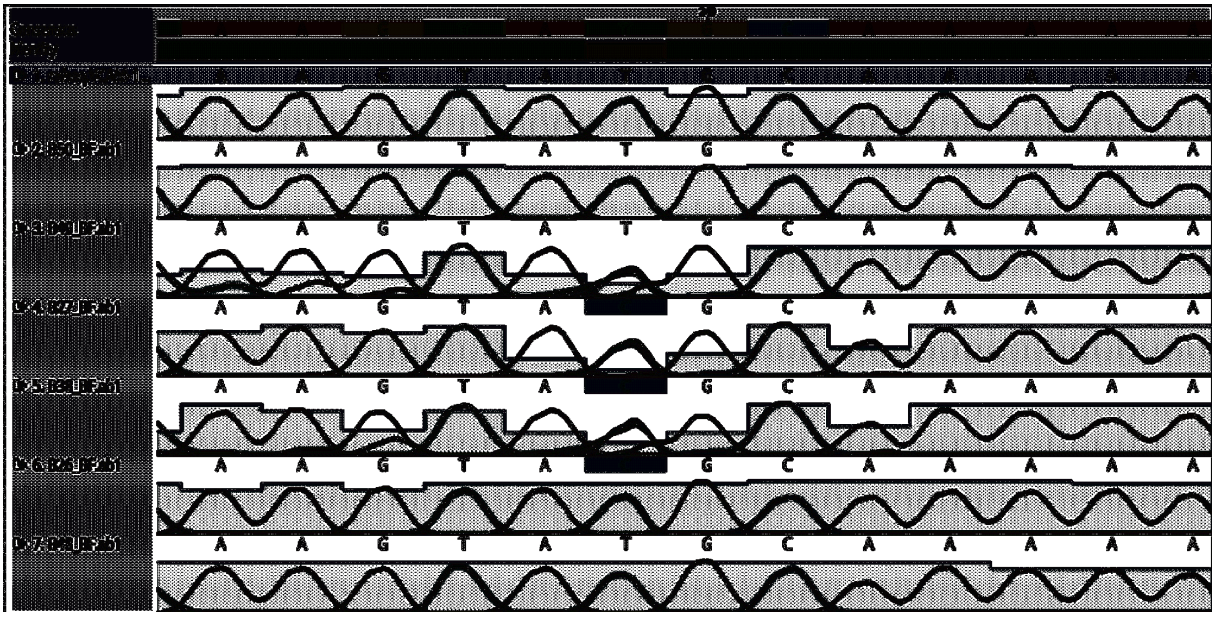

Figure 2: Comparison of sequence alignment of a portion of the VCAM-1 gene in the promoter between rheumatoid arthritis patients and healthy controls showing rs3170794.

Table 2: Genotypes distribution and allele frequencies (observed and expected) of VCAM-1 gene (rs3170794) and their (HWE) in rheumatoid arthritis patients and control.

Genotype rs3170794	RA Patients (N=30)				Controls (N=20)			
	Observed		Expected		Observed		Expected	
	No.	%	No.	%	No.	%	No.	%
Genotype (TT)	27	90	25.21	84.03	19	95	19.01	95.06
Genotype (CT)	1	3.33	4.58	15.28	1	5	0.98	4.88
genotype (CC)	2	6.67	0.21	0.69	0	0.0	0.01	0.06
Alleles								
allele T	55	91.67	Not Estimated		39	97.5	Not Estimated	
allele C	5	8.33			1	2.5		
Chi-square test results	0.001				0.9087NS			
Outcome	The population is in Hardy-Weinberg equilibrium							

NS: non-significant (p≥0.05)

Table 3: Statistical analysis and Genotypes distribution and allele frequencies of rs3170794 in patients and control

Genotype rs3170794	Study groups		Percentage of (EF) and (PF)	OR	Fisher's Exact probability	95% CI
	RA Patients (N=30)	Controls (N=20)				
TT n(%)	27(90)	19 (95)	%52.6	0.47	0.465NS	0.02 - 4.86
CT n(%)	1 (3.33)	1(5)	%34.5	0.66	0.755NS	0.02 - 26.84
CC n(%)	2 (6.67)	0(0.0)	%5.8	3.60	0.333NS	0.18 - 73.61
Allele						
T n(%)	55 (91.67)	39 (97.5)	%71.8	0.28	0.306NS	0.01 - 2.16
C n(%)	5 (8.33)	1 (2.5)	%71.8	3.55	0.306NS	0.46 - 86.37

NS: non-significant (p≥0.05).

A higher frequency of the T allele was observed in the control group (97.5%) compared to the patient group (91.67%), although this difference was not statistically significant ($X^2 = 0.306$, OR = 0.28, 95% CI: 0.01 - 2.16). Conversely, the C allele exhibited a greater frequency in patients (8.33%) than in controls (2.5%), also without reaching statistical significance ($X^2 = 0.306$, OR = 3.55, 95% CI: 0.46 - 86.37).

Although not statistically significant, the observed odds ratios suggest that the T allele may have a protective effect, whereas the C allele may represent a potential genetic risk factor for rheumatoid arthritis. These findings should be interpreted with caution because of the limited sample size.

3 DISCUSSION

Cell adhesion molecules (CAMs), like VCAM-1, are major participants in rheumatoid arthritis (RA). They facilitate the adhesion of white blood cells to the vessel walls and their migration into the inflamed synovial tissue, thus contributing to the already existing chronic inflammatory process. More recently, VCAM-1 is also found not only to be involved in the local inflammatory process but also in vascular endothelium activation and cardiovascular risk factors associated with RA [20], [21]. This is an example of how genetic studies can prove that single nucleotide polymorphisms can have an impact on more than just a single disease but can actually touch on all aspects of health. However, the differences that have been identified across various ethnic groups also suggest that genetic factors, environmental factors, and lifestyle can all play a role in the level to which single nucleotide polymorphisms actually contribute to the development of a disease [22], [23]. In this study, it is found that the TT genotype is the most common in both patients and controls, as shown in Table 2. Moreover, the C allele decreases in frequency, and the CC genotype is only found in patients and not controls. In both groups, the Hardy-Weinberg equilibrium is satisfied; thus, the results are more reliable because both groups are genetically stable. As shown in Table 3, it is found that the CC genotype has a fairly high odds ratio, which is 3.60, and the C allele is close to this, with an odds ratio of 3.55, which is trending towards increased risk; and the TT genotype has a low odds ratio, which is 0.47, and the T allele suggests it could be protective, with an odds ratio of 0.28. However, none of these results is statistically significant, due to the small sample size. Therefore, it is found that there is no statistically

significant relationship between the rs3170794 polymorphism in the VCAM-1 gene and rheumatoid arthritis risk, are consistent with some previous studies that investigated VCAM-1 polymorphisms in different disease contexts. The research done by Domanski and his co-authors has shown that the VCAM-1 gene (identified as containing the rs3170794 polymorphism), was not related to pathological changes found in transplanted kidney biopsies. These results suggest that this polymorphic variant may not have a strong or direct influence in certain inflammatory conditions [16].

Also, the CC genotype has a fairly high odds ratio, though not statistically significant, of 3.60. This implies that perhaps a larger sample size is necessary. Often, genetic studies to identify associations with rare genetic variants for complex diseases require a larger sample size, for example, genome-wide association studies to identify small to moderate effects, avoid errors, and limit selection or genotyping biases [24]. The small sample size in this study (N=50) may have limited the statistical power to detect a true association. Although the results of this study did not find a direct genetic link between the rs3170794 polymorphism and RA, these results do not contradict the established biological role of VCAM-1 in the disease. Another recent study, conducted recently, focused on endothelial markers, like VCAM-1, and made an association between them and imaging and inflammation markers. The study found that it appears VCAM-1 may be an endothelial/inflammatory dynamic marker, rather than being a static genetic determinant of disease [21]. Another study conducted in 2022 found that elevated levels of VCAM-1 may be used as an indicator for identifying interstitial lung diseases in RA [25].

Biologically, the regulation of VCAM-1 expression is complex and involves multiple signaling pathways, but basically, it's mainly controlled by inflammatory pathways, particularly the NF-κB pathway, which is activated by cytokines like TNF-α and IL-1β. Previous studies have demonstrated that the VCAM-1 promoter indeed harbors regulatory regions that are highly responsive to this pathway, and hence, the effect of a single nucleotide polymorphism within this promoter region would not have a great impact unless it occurs with other genetic or environmental factors. [26]-[30]. Furthermore, there are already some genetic studies in other inflammatory conditions showing that the role of VCAM-1 polymorphisms varies by ethnicity and even by type of condition. Previous studies in obesity, diabetes, and organ transplantation suggest that these polymorphisms may act as disease modifiers rather than direct risk factors [15], [16]. This proposition is appropriate for explaining the results of the current study in the Iraqi community, where linkage disequilibrium and allele frequencies may differ from those in other communities.

4 CONCLUSIONS

This study found no statistically significant association between the VCAM-1 rs3170794 polymorphism and rheumatoid arthritis in the Iraqi population. Nevertheless, the observed genotype distribution suggests that the C allele may represent a potential genetic risk factor, whereas the T allele may have a protective effect against disease development. Although these findings require further confirmation, they contribute to the understanding of the possible genetic involvement of VCAM-1 in rheumatoid arthritis. Future investigations incorporating larger cohorts, diverse populations, environmental risk factors, and functional biomarkers, including soluble VCAM-1 levels and disease activity indicators, are needed to clarify the biological role of this polymorphism in rheumatoid arthritis pathogenesis.

5 LIMITATIONS

The present study has several limitations. First, the relatively small sample size (n = 50) limited the statistical power of the analysis, resulting in wide confidence intervals and reduced precision of the estimated association between the CC genotype and rheumatoid arthritis susceptibility. Second, only a single VCAM-1 polymorphism (rs3170794) was investigated, whereas additional genetic variants and functional mechanisms were not assessed. Therefore, the findings should be considered preliminary and interpreted with caution. Future multicenter studies involving larger cohorts and comprehensive genetic and functional analyses are required to validate these observations.

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