Study of some genetic and molecular markers for some rheumatoid arthritis patients in Iraq.

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Abstract
Rheumatoid arthritis is an autoimmune disorder, and genetic factors strongly contribute to a genetic predisposition to developing the disease. This study evaluated the genetic and molecular indicators of some Iraqi patients with rheumatoid arthritis. The study included (100) patients with rheumatoid arthritis with (100) healthy individuals who attended Al-Hussain General Teaching Hospital, Department of Arthritis and Joints Centre, al Blood Bank in Baghdad for the period from the beginning of January 2022 until the end of March 2022. The patients were diagnosed under the supervision of medical committees specialized in joint diseases. The human leukocyte antigen is one of the essential genetic factors in regulating the immune response, as these antigens contribute to the susceptibility to disease. Human leukocyte antigen (HLA) class II (Class-II- HLA-DR, -DQ) was genotyped using lymphocytotoxicity assay and PCR-SSP method. The results showed that there was a significant increase in the recurrence of human leukocyte antigens (DR4 R53) in rheumatoid arthritis patients compared to the healthy ones, as well as an increase in the recurrence of human leukocyte antigens (HLA-DQ3) with a significant difference in rheumatoid arthritis patients compared to the healthy ones. Regarding HLA-DRB1 and -DQB1 alleles, it was found that there was a significant increase in the frequency of HLA-DRB1*04 (01-22, not 0415) compared to healthy controls, while the percentage of HLA-DRB1*0701 alleles was less frequent in patients compared to healthy controls. Moreover, the frequency of HLA-DQB1*03(02,07) alleles was high in the patients compared to the healthy ones, while HLA-DQB1*0303 showed a highly significant difference in the healthy group compared to the patients.

Keywords: Rheumatoid arthritis, genetic factors, HLA-DRB1, -DQB1 alleles, PCR.

Introduction
Rheumatoid arthritis (RA) is a multifaceted inflammatory disease with a significant hereditary component to its pathophysiology. Most of the population is vulnerable to this illness due to genetic factors. Predisposition to RA has been demonstrated to be influenced by genetic variables. Variation (or polymorphism) of the genes encoding numerous proteins known to be highly implicated in driving the inflammatory process in RA has been discovered in studies. The existence of genetic risk factors is supported by the fact that the prevalence
of RA differs amongst groups. They are crucial in explaining illness risk disparities. It also reflects the fact that various populations have diverse lifestyles, diets, smoking behaviors, and other factors that may increase RA susceptibility. First, according to genetic predisposition studies in families, degree RA patients get the illness at a greater incidence than the general population. This suggests a hereditary propensity to the disease. The absence of a Mendelian inheritance pattern shows numerous genetic components. An essential characteristic of HLA antigens is the assistance of linkage disequilibrium between the loci alleles. It is an unexpected association of linked genes between the alleles at one HLA locus with a given allele at a second HLA locus in the population (Appendix I). It varies with the ethnic group to the extent that the association between two or more antigens can be a character of a particular ethnic group. Linkage disequilibrium is believed to be the result of genetic drift or migratory admixture. Linkage Disequilibrium is a hallmark of human MHC and extends from HLA-A through HLA-DQ.

Linkage studies are key categories of research aimed at determining the role of genes in disease susceptibility. When only family data may be used in linkage research, association studies might be family or population-based. The ultimate goal of HLA association studies is to find out how genes cause the disease or modify susceptibility or course of it. The association may result from direct involvement or linkage disequilibrium with the gene at the population level. HLA molecules are thought to function in autoimmune disease pathogenesis. In 1973, ankylosing spondylitis was linked to the HLA-B27 gene. This was followed by describing the association between several class II MHC molecules and autoimmune diseases, including RA, Insulin-dependent Mellitus, and Multiple Sclerosis. There is also good evidence that HLA molecules influence host defense against foreign pathogens, allergic responses and antitumor immunity. The mechanisms of HLA association with the disease have yet to be adequately explained. Most studies that agree with the association are usually described in terms of susceptibility, while negatively associated molecules are sometimes referred to as protective. The problem is strong linkage disequilibrium or nonrandom association between genes in the HLA complex, creating significant differences when establishing which HLA genes are primarily involved. International collaboration through histocompatibility workshops (has been very important in addressing this question since HLA gene linkage disequilibria vary between populations and ethnic groups. Thus, comparing the HLA as an association with disease in different populations greatly facilitates identifying the primarily involved HLA genes. A number of studies have been proposed to explain the HLA-disease link based on current knowledge of the biology of the HLA molecule. Despite more than a quarter-century of research, the mechanisms of HLA participation in autoimmune disorders remain unknown. This is because of the difficulties of identifying the influence of a specific HLA molecule on the illnesses under investigation. This issue has surfaced in examining illnesses using epidemiological, genetic, and immunological methods. Some of the reported studies which have been done in Iraq on the association of HLA and diseases such as Systemic lupus erythematosus (SLE) were associated with A1, A2, B8, 3 and Rheumatoid arthritis was associated with A10, B47, B22, Cw 7, DR4, DR52, DR53 and DO3. Rheumatoid arthritis is an autoimmune illness with several causes. The most significant HLA genes have an impact on illness vulnerability. Gregersen and coworkers devised a formula in 1987. The shared epitope (SE) theory is based on the discovery that disease-associated HLA-DRB1 alleles (DRB1*0101, *0401, *0404, *0405, and *0408) have a similar amino acid sequence. DRB1*1001 has also been found in common epitope
alleles, which might explain why DRB1*1001 is linked to rheumatoid arthritis in different groups. On the other hand, HLA class II alleles or haplotypes appear to confer protection against RA in addition to susceptibility genes. In the year 2000, Zanelli and his colleagues developed the RAP (rheumatoid arthritis protection) model. According to this, the haplotypes HLA-DQA1*01-DQB1*0501 (DQ5) (linked to DRB1*0101, *0102, *0103, and *1001) and DQA1*03-DQB1*03 (DQ3) (linked to RB1*0901 or any DRB1*04 allele) predispose to rheumatoid arthritis, while the DRB1 alleles *24-31. The HLA is a genetically controlled molecule that captures antigen fragments and displays them on the surface of cells for antibody and T-cell destruction. It has made to tell the difference between self and non-self cells. For almost 30 years, researchers have identified a variety of HLA genetic forms known as HLA DRB1 alleles as a risk factor for RA, with the HLA DRB1*0401 and DRB1*0404 alleles being considerably greater susceptibility factors than the HLA DRB1*0101 alleles. The HLA DRB1*0401 and DRB1*0404 alleles contain specific amino acid sequences in the same three-dimensional epitope's peptide-binding groove, known as the common epitope (SE). Both susceptibilities A and the severity of the illness are influenced by the presence of HLA DRB1/SE, particularly the development of erosions and the incidence of extra-articular RA. The antigen presentation to T-cell receptors activity of the HLA DRB1/SE is important in RA. HLA-DP in individuals with Anti-cyclic citrullinated peptide antibodies is another locus revealed by high-density genotyping contributing to the risk of rheumatoid arthritis.

Materials and Methods
DNA Extraction • HLA-DRB1 and DQB1 (SSP-PCR) Typing PCR-SSPs (Polymerase Chain Reaction Sequence-Specific Primers):
Purified DNA is the starting point for typing using the HISTO TYPE/DNA-SSP kit. The Sequence Specific Primers (SSP) were used in the test process (Figure 1). This approach is predicated on the notion that primer extension, and therefore effective PCR, requires a precise match at both primers’ 3’ ends.

Figure 1. Sequence-specific primers in the polymerase chain reaction (PCR-SSP).

Statistical analysis
Statistical analysis was done using the software SPSS version 2020. The results were expressed as mean ± standard deviations (mean ± SD). Oway ANOVA-test and Chi-square were used to compare parameters in different studied groups. P-values (P ≤ 0.05) were considered statistically significant.

Results
As a result, the amplification produced is only seen by agarose gel electrophoresis if the primers match entirely the target sequence. The composition of each primer mixture allows for easy identification of the HLA types shown in the assessment diagrams.
Contents of the PCR-SSP tray (KKU HLA PCR-SSP Kits, KKU).
The PCR trays consisting of several PCR semi-tubes, twenty semi-tubes for each oofDRB1 and seventeen for HLA-DQB1, were available in the kit. Each semi-tube contains the prop-er-pre-dropped-protection allele-specific primers and internal control primers (specific for the human growth hormone (HGH) and nucleotides).

HLA-Genotyping
In the PCR-SSP method, we used 20 HLA-DR and 17 HLA-DQ specific primers mixture and ladder mixture. This system was utilized to determine the alleles of individual HLA-DR and DQ alleles in patients with RA and healthy controls. In all lanes except the opposing control lane, where no amplification occurred, an upbeat internal control band was generated due to successful amplification. An internal positive control band and an upbeat particular amplification band were generated as a result of the positive specific amplification see (Figures 2 and 3).

Figure 2. Electrophoresis of HLA-DR and HLA-DQ alleles amplification (1% agarose gel, for 20 min at 200-volt volts PCR-SSP of RA Patients.

*First line (1-15), and third line (16-20) represent the HLA-DR genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1= 1R; 2= 2R; 3= 3R; 4= 4R; 5= 5R; 6= 6R; 7= 7R; 8=8R; 9= 9R; 10= 10R; 11= 11R; 12= 12R; 13=13R; 14= 14R; 15= 15R; 16=16R; 17 = 17R; 18= 18R; 19= 19R; 20= 20R. (More details can be found in the result sheet in Appendix II-A).

*Second line(1-15), third line (16-17) represent the HLA-DQ genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1= 1Q; 2= 2Q; 3= 3Q; 4= 4Q; 5= 5Q; 6= 6Q; 7= 7Q; 8=8Q; 9= 9Q; 10= 10Q; 11= 11Q; 12= 12Q; 13=13Q; 14= 14Q; 15= 15Q; 16=16Q; 17 = 17Q (More details can be found in the result sheet in appendix II-B).
Figure 2. Electrophoresis of HLA-DR and HLA-DQ alleles amplification (1% agarose gel, for 20 min at 200 volts) PCR-SSP of RA Patient.

*First line (1-15), third line (16-20) represent the HLA-DR genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1= 1R; 2= 2R; 3= 3R; 4= 4R; 5= 5R; 6= 6R; 7= 7R; 8=8R; 9= 9R; 10= 10R; 11= 11R; 12= 12R; 13=13R; 14= 14R; 15= 15R; 16=16R; 17 = 17R; 18= 18R; 19= 19R; 20= 20R. (More details can be found in the result sheet in Appendix II-A).

*Second line (1-15), third line (16-17) represent the HLA-DQ genotyping was obtained by using primers that detect the following alleles as they were present in the numbered wells, respectively: 1= 1Q; 2= 2Q; 3= 3Q; 4= 4Q; 5= 5Q; 6= 6Q; 7= 7Q; 8=8Q; 9= 9Q; 10= 10Q; 11= 11Q; 12= 12Q; 13=13Q; 14= 14Q; 15= 15Q; 16=16Q; 17 = 17Q (More details can be found in the result sheet in appendix II-B).

Association of HLA-class II Alleles with RA

When it comes to rheumatoid arthritis, it is the HLA area that has the most impact on the likelihood of developing the disease. Some experts believe that genetic factors account for between 50 and 60 percent of the chance of developing rheumatoid arthritis. One of this investigation’s objectives was to determine the relationship between the prevalence of RA and certain HLA-DR and DQ alleles. HLA allele frequencies in patients and controls were compared. Frequency distributions were developed to reveal whether the HLA-DR and DQ alleles were worn or less common in 100 patients and controls.

According to the statistical study of the DR locus, only the DR *04 (01-22, not 0415) alleles were substantially more common than healthy controls. However, the adjusted values over loop values were significant (P<0.001). Due to their heightened relative risk (RR) of 52.3% and an etiological fraction (EF) of 38.8%, these fre-
quencies are associated with an increased disease risk. In contrast, the DR* 07al-
lele sele had a much lower frequency in RA compared to the healthy controls. As 
well as the following alleles, DR*01 (01, 02, 04) and DR*13 (01, 05, 06, 09, 10, 16, 18, 20, 27, 88, 31) were presented with higher 
frequencies in RA patients than healthy control group with RR (2.43; 4.75) and 
EF (0.17;0.15) respectively, but it did not reveal any significance (table 1), while 
the DR*15(01-03,06), DR*12(01,03,05), DR*08(01-19, not 0805,0818), 
DR*0415, DR*1001 allele were showed significant increased frequency in healthy 
controls more than RA patients with P (0.023; 0.021; 0.186; 0.021) respectively. 
Among DQ- locus alleles, the DQB1*03 (02, 07) alleles revealed higher frequen-
cies in RA patients than in healthy control with RR (3.69) and EF (0.34). In com-
parison, DQB1*0303 alleles showed higher frequencies in the healthy control 
group than RA patients with highly significant (P<0.001) with RR (0.11) and PF 
(0.35), which were mentioned in table (2).
In addition, the comparison with healthy control: some alleles DQB 0301 were 
increased in RA more than the control group but statistically not significant as well 
as the following alleles: QB10401; DQB1*06(02,10,11,13), which were presented 
with higher frequencies in healthy cont. than RA patients with RR (0.52) and PF 
(0.11), while DQB1*02(01,02) only found the n healthy group, but it did not 
show any significance.

<table>
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<tr>
<th>HLA-DRB1</th>
<th>RA (100)</th>
<th>Control (100)</th>
<th>RR</th>
<th>EF</th>
<th>PF</th>
<th>P-value</th>
<th>PC</th>
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<tbody>
<tr>
<td>Genotype</td>
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<td>%</td>
<td>N</td>
<td>%</td>
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<td>30</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>2.43</td>
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<td>DR*03(01,06,08,10)</td>
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<td>12</td>
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<td>48</td>
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<td>5</td>
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<td>15</td>
<td>0.298</td>
<td>0.117</td>
<td>0.105</td>
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<tr>
<td>DR *0415</td>
<td>3</td>
<td>3</td>
<td>20</td>
<td>20</td>
<td>0.102</td>
<td>-0.218</td>
<td>0.179</td>
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<tr>
<td>DR*1001</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>15</td>
<td>0.298</td>
<td>-0.117</td>
<td>0.105</td>
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<tr>
<td>DR*11(01-31 not 11(09,10,13,16,17,20, 22)</td>
<td>25</td>
<td>25</td>
<td>15</td>
<td>15</td>
<td>1.889</td>
<td>0.117</td>
<td>-0.133</td>
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<tr>
<td>DR*12(01-03,05)</td>
<td>3</td>
<td>3</td>
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<td>20</td>
<td>0.123</td>
<td>-0.213</td>
<td>0.174</td>
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<tr>
<td>DR*13(01,05,06,09,10,16,18,20, 27,28,31)</td>
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<td>DR *14(02,06,19,20)</td>
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<td>5</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DR *1302</td>
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<td>3</td>
<td>20</td>
<td>20</td>
<td>0.102</td>
<td>-0.218</td>
<td>0.179</td>
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</table>
Table 1. Compared with healthy controls, the frequency and (RR, EF, PF) of the HLA-DR alleles in RA patients.

<table>
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<tr>
<th>Genotype</th>
<th>N</th>
<th>%</th>
<th>N</th>
<th>%</th>
<th>RR</th>
<th>EF</th>
<th>PF</th>
<th>P-value</th>
<th>PC</th>
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<td>DQB1*0501</td>
<td>20</td>
<td>20</td>
<td>15</td>
<td>15</td>
<td>1.417</td>
<td>0.058</td>
<td>-0.062</td>
<td>0.637</td>
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<td>DQB1*0601</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
<td>NS</td>
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<tr>
<td>DQB1*06(02,10,11,13)</td>
<td>2</td>
<td>2</td>
<td>15</td>
<td>15</td>
<td>0.115</td>
<td>-0.153</td>
<td>0.129</td>
<td>0.067</td>
<td>NS</td>
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<td>DQB1*02(01,02)</td>
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<td>0</td>
<td>10</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0.042</td>
<td>NS</td>
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<tr>
<td>DQB1*0301</td>
<td>35</td>
<td>35</td>
<td>10</td>
<td>10</td>
<td>4.846</td>
<td>0.278</td>
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<td>0.039</td>
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<tr>
<td>DQB1*03(02,07)</td>
<td>48</td>
<td>48</td>
<td>20</td>
<td>20</td>
<td>3.692</td>
<td>0.349</td>
<td>-0.538</td>
<td>0.00</td>
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<tr>
<td>DQB1*0303</td>
<td>7</td>
<td>7</td>
<td>40</td>
<td>40</td>
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<td>-0.555</td>
<td>0.356</td>
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<td>DQB1*0401</td>
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<td>25</td>
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<td>0.529</td>
<td>-0.133</td>
<td>0.117</td>
<td>0.345</td>
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Table 2. Compared with healthy controls, the frequency and (RR, EF, PF) of the HLA-DQ alleles in RA patients.

Discussion

Rheumatoid arthritis (AR) is a common systemic autoimmune disorder characterized by chronic, symmetric, and erosive arthritis of the peripheral joints, affecting men and women of all ages, with a peak incidence in premenopausal women.

Rheumatoid arthritis is a disease of multifactorial origin, including a genetic predisposition, characterized by immune-driven, chronic inflammation marked by a variable course involving exacerbations and remissions of disease activity. One of the aims of this research was to determine whether there was a link between the frequency of RA and various HLA-DR and DQ alleles. The frequencies of HLA alleles in patients and controls were compared. In 100 patients and controls, frequency distributions were created to see if the HLA-DR and DQ alleles were more or less prevalent. Only the DR *04 (01-22, not 0415) alleles were significantly more prevalent than healthy controls in a statistical examination of the DR locus. However, the adjusted values over loop values were significant (P<0.001). These frequencies are connected with an elevated risk of disease due to their higher relative risk (RR) of 52.3% and an etiological fraction (EF) of 38.8%. In contrast, the DR* 07 allele was substantially less common in RA than in healthy individuals. DR*01 (01, 02, 04) and DR*13 (01, 05, 06, 09, 10, 16, 18, 20, 27, 88, 31) alleles, as well as the following alleles, were found to be more common in RA patients than in the healthy control group, with RR (2.43; 4.75) and EF (2.43; 4.75) being the most common (0.17;0.15) respectively. Among DQ-local alleles, the DQB1*03 (02, 07) allele was more common in RA patients than in healthy controls with RR (3.69). (0.34). In comparison, DQB1*0303 alleles were found at a greater frequency in the healthy control group than in RA patients, with very significant (P<0.001) RR (0.11) and PF (0.35), as shown in table (2).
Furthermore, some alleles DQB 0301 were increased in RA more than the control group but were not statistically significant, as were the following alleles: QB10401; DQB1*06(02,10,11,13) which were presented with higher frequencies in healthy control than RA patients with RR (0.52) and PF (0.11), while DQB1*02(01,02) only found the n healthy group but did not show any significance. In this regard, no work has attempted to identify a link between HLA genotyping and RA in Iraqi patients. To evaluate the function of HLA-DR and DQ in RA immunobiology, we used PCR-SSP to describe their genotypes. In this study, the DR*04 (01-22, not 0415) allele was shown to have a highly significant correlation with RA patients (P= 0.000) when compared to healthy controls. The RR for this allele was (5.23), meaning that people with this allele have a 5.23 times greater likelihood of developing RA than those who do not have it. This finding is consistent with van der Woude and van der Helm-van 46, who found statistically significant increased DRB1*04 alleles (DR4) in Hungarian patients compared to healthy subjects, and Regueiro et al. 47, who explained that DRB1*04 alleles (DR4) were associated with RA, with the DRB1*0404 alleles having the most substantial effect. Anaya et al. 48 found a similar link between DR4 and RA patients among Colombian patients. However, Ash and Roy et al., 49 found no statistically significant difference in DRB1*04 allele frequencies between RA patients and controls. As a result, the RR for people with the DRB1*04 alleles (DR4) is around 6 times greater than for people who do not have the alleles 43-47. Many studies confirmed that the HLA-DRB1*04 alleles (DR4) were strongly associated with RA patients in cases studied by 50, particularly in South Asian countries including India 53-55, while in Iraq Correspondance each 51-54, with but Caucasian RA subtypes of DR1 and DR4 occur more frequently than subtypes of DR10 or DR14 55. The prevalence of DR4 is very high among RA patients in the general population 55-57. All of the studies show that the connection of a specific allele with RA differs by race, implying that the frequencies of HLA-DR and HLA-DQ alleles in Iraqi RA patients should be investigated. These genetic variations in HLA-DRB1 and other nearby loci have been linked to early RA therapy response 34. Compared to healthy controls, the DR* 0701 all alleles have a much lower frequency in RA, suggesting that these alleles may have protective benefits against the illness. DR*01(01,02,04); DR*13(01,05,06,09,10,16,18,20,27,28,31) ; DR*11(01-31 not 11(09,10,13,16,17,20,22) ; DR*11(01-31 not 11(09,10,13,16,17,20,22) ; and DR*11(01-31 not 11(09,10,13) Other studies have revealed that DR*01 is more prevalent in RA than in controls 51-58, while Constantinidou et al. (1998) discovered a substantial relationship between RA and DR*01 in the Greek population. This finding goes with 27-32. Although the frequency of DR*15(01-03, 06) alleles was higher in healthy controls than in patients, the difference was not statistically significant, suggesting that these alleles may have disease-protective effects. In contrast, a prior study found a high frequency of DR*15 in RA patients of Pakistani ancestry 59. However, the current findings support an Indian study that found a low frequency of DR*15 in Indian patients compared to RA-free controls 14-17. According to these findings, the
DR*15 gene may have varied associations with RA in various groups. According to many studies, the DRB1 locus is responsible for 30% of the hereditary risk of RA. These findings support previous findings that severe RA has a significant link to HLA-DR4 and that people with severe RA have a higher risk of developing the condition. Only HLA-DRB1 alleles have been linked to and associated with RA, meeting the criteria for a fully demonstrated genetic factor. These molecules are prominent in the genetic risk of developing RA.

Furthermore, when RA patients were compared to control groups, no statistically significant high frequencies of some HLA-DQ alleles were found, such as DQB1*0301, whereas 03 (02,07) exhibited extremely significant frequencies with RR(3.69), EF(0.34), and DQB1*0301 with a high RR (4.84). The frequency of DQB1*0303 alleles in RA patients was considerably lower than in healthy controls. This allele might provide the foundation for disease resistance, but further research is needed to confirm this link. Our findings were consistent with those in Indian research, which found a high frequency of DQ3 in Indian RA patients and a link between DR4 and DQ3, as well as others, who found that DQ3 had the most significant frequencies among RA patients in north-east England. Also, using serological approaches, Albarzinji, et al. found a link between DR4 and DQ3 and illness. These genetic variations impact disease manifestation, and it has been postulated that not all alleles encoding the common epitope are comparable in terms of their connection with RA susceptibility or severity when present as a single allele or as a pair of alleles. Other researchers have reported that this dosage does not appear authentic in all ethnic groups; for example, most African-American RA patients do not exhibit the common epitope, and disease severity is identical for those with and without the epitope, regardless of allele dose. According to a few studies, DQB1 alleles may alter the clinical or biological manifestation of the illness due to a complimentary action of the DRB1 and DQB1 genes. From the result findings of this study, there are high frequencies of HLA-DR, HLA-DR53 and HLA-DQ3 among RA patients. This emphasizes the role of HLA-DR antigen in rheumatoid arthritis susceptibility.

Based on the current data, the frequency of the HLA-DRB1*04 (01-22, not 0415) allele was significantly increased in RA patients compared to the healthy control group. This may indicate the presence of at least one necessary genetic factor for disease susceptibility, while the HLA-DRB1*0701 allele showed significantly low frequency in patients compared with the control group. The frequency of HLA-DQB1*03(02,07) was significantly increased in RA patients compared with healthy control group control groups, whereas HLA-DQB1*0303 was presented with higher frequency in healthy than RA patients.

**Conclusions**

Regarding HLA-DRB1 and -DQB1 alleles, it was found that there was a significant increase in the frequency of HLA-DRB1*04 (01-22, not 0415) compared to healthy controls, while the percentage of HLA-DRB1*0701 alleles was less frequent in patients compared to healthy controls. Moreover, the frequency of HLA-DQB1*03(02,07) alleles was high in the patients compared to the healthy ones,
while HLA-DQB1*0303 showed a highly significant difference in the healthy group compared to the patients.

**Recommendation**
Based on the findings of the present study, the following recommendations can be suggested:
Further studies are required to clarify the role of HLA class I and HLA class III in susceptibility and development of disease and the Familial study of HLA–genotyping for patients. Studying other parameters to compare between groups, for example, following up the level of immunity specific to antibodies, some types of Interleukins, and specialized parameters in all studied groups.

**Compliance with Ethical Standards statements**

**Ethical approval:**
The manuscript is written in original, and all the data results about this manuscript are original according to the research performed. The authors followed academic integrity and did not copy any content/results from another source.

**Funding details (In case of Funding):** The authors of this manuscript did not receive any funding to perform the present research

**Conflict of interest:** The authors of the study do not have any conflict of interest

**Informed Consent:** The manuscript's authors agree to publish this research in the journal if the journal editors approve it. The authors provide full consent for reviewing and publishing this manuscript.

V. All the authors of this study contributed equally in terms of performing the research and preparing the manuscript. All the authors of the study followed the guidelines of the corresponding author. Any query/suggestion related to the manuscript can be sent to the corresponding author

**Acknowledgments:** We thank the General Directorate of Education in Thi-Qar for completing this study.

**References**


19. Clanchy, F. I., Borghese, F., Bystrom, J., Balog, A., Penn, H., Taylor, P. C., ... & Williams, R. O. Disease status in human and experimental arthritis, and response to TNF blockade, is associated with MHC class II invariant chain (CD74) isoform expression. Journal of Autoimmunity, 2022; 128, 102810.


42. Padyukov, L. Genetics of rheumatoid arthritis. In Seminars in Immunopathology, 2022; (pp. 1-16). Springer Berlin Heidelberg.


58. Yang, Z., Liu, W., Yan, T., & Liu, R. HLA-DPB1 rs9277535 polymorphism is associated with rheumatoid arthritis risk in a Chinese Han population. Aging (Albany NY), 2021; 13(8), 11696.


60. Zhang, T. Perinatal risk factors for mental disorders in the offspring and in their mothers, 2022.