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HLA genotyping in cardiac and other extrapulmonary manifestations of sarcoidosis

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Abstract

Background: Although numerous candidate genes have been identified in studies investigating the role of genetics in sarcoidosis, the strongest association has been reported with the Major Histocompatibility Complex/Human Leucocyte Antigen (MHC/HLA) region. This study aimed to evaluate HLA polymorphism and assess its association with cardiac and other extrapulmonary involvement in sarcoidosis patients.

Methods: The study included 67 patients diagnosed with sarcoidosis. A control group of 100 bone marrow donors, who had undergone HLA genotyping previously, was also included. Blood samples were collected from all participants for HLA gene polymorphism analysis. The differences in HLA genotypes were investigated between patients with and without cardiac and other extrapulmonary involvement, and between these groups and the control group.

Results: Cardiac involvement, was present in 17.9% of the patients. The most frequently affected extrapulmonary organ was the skin (23.8%). HLA DQB103 and HLA DQB106 alleles were expressed more frequently in patients with only pulmonary involvement compared to those with extrapulmonary involvement. Conversely, HLA DQA101 was expressed more frequently in patients with extrapulmonary involvement. No statistically significant difference in the expression of HLA DRB1, HLA DQB1, and HLA DQA1 alleles was observed between sarcoidosis patients with and without cardiac involvement.

Conclusion: Our findings suggest that HLA DQB103 and HLA DQB106 alleles might be protective against extrapulmonary organ involvement, while HLA DQA101 could be a risk factor. These findings may contribute to the prediction of treatment response and prognosis in sarcoidosis patients.

Abbreviations: 18FDG-PET/CT = 18-fluorodeoxyglucose positron emission tomography/computed tomography, ACCESS = A Case Control Etiologic Study of Sarcoidosis, BAL = bronchoalveolar lavage, BAP = Scientific Research Projects Unit, CI = confidence interval, DLCO = Diffusing capacity of the lung for carbon monoxide, DLCO = diffusing capacity of the lung for carbon monoxide, DLCO/VA = DLCO/alveolar ventilation ratio, EBUS-TBNA = Endobronchial ultrasound-guided transbronchial needle aspiration, ECG = electrocardiography, ECHO = echocardiography, EMB = endomyocardial biopsy, FEF25-75 = forced expiratory flow 25% to 75%, FEV1 = forced expiratory volume in 1 second, FVC = forced vital capacity, MHC/HLA = Major Histocompatibility Complex/Human Leucocyte Antigen, MR = magnetic resonance, OR = odds ratio, PA = posteroanterior, PFT = pulmonary function tests.

Keywords: cardiac involvement, extrapulmonary involvement, genotyping, HLA polymorphism, Sarcoidosis

1. Introduction

Sarcoidosis is a multisystemic granulomatous disease of unknown etiology, most commonly affecting the lungs and intrathoracic lymph nodes.[1] While it can occur worldwide, in individuals of any age, sex, or race,[2] the most common age of onset is between the second and fourth decades of life, with a second peak incidence in women over 50.[3] The involvement of organs exposed to the environment, such as the skin, eyes, and lungs, suggests a potential role for environmental agents in the etiology of sarcoidosis.[4] However, a familial predisposition to sarcoidosis is also recognized. Familial sarcoidosis was first reported in 2 affected sisters in 1923.[5] One of the most significant studies in this area, the A Case Control Etiologic

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Study of Sarcoidosis (ACCESS), found a 4.7-fold increased risk of sarcoidosis in first- and second-degree relatives of affected individuals compared to controls. Siblings had the highest relative risk (odds ratio 5.8), followed by aunts/uncles (5.7) and grandparents (5.2). White individuals had a significantly higher familial relative risk compared to African Americans (18.0 vs. 2.8).[6]

While numerous candidate genes have been identified in studies investigating the role of genetics in sarcoidosis, the strongest association has been reported with the Major Histocompatibility Complex/Human Leucocyte Antigen (MHC/HLA) region.[7,8] The first report described an association between acute sarcoidosis and the specific gene product, class I HLA-B8 antigen.[9] A strong association has been established between HLA class II antigens, encoded by the HLA-DRB1 and DQB1 alleles, and sarcoidosis.[10,11] Another study related to HLA found that while certain alleles increase the risk of sarcoidosis, other alleles are associated with specific phenotypes, such as Löfgren syndrome.[7] Grunewald et al suggested that the HLA DRB103 allele is associated with a good prognosis, whereas the HLA DRB115 allele is associated with a poor prognosis.[12] Naruse et al reported an association between HLA DQB10601 and cardiac sarcoidosis in a Japanese population.[13] However, sufficient data and studies are lacking to demonstrate the association between HLA and cardiac/non-cardiac sarcoidosis in the Turkish population.

Some studies have suggested that HLA genotypes contribute more to disease susceptibility than to the specific clinical presentation; for example, HLA-DQB10201 and 0301 have been linked to acute onset and good prognosis.[14] The low prevalence of some alleles, such as HLA-DRB101 and 04, in sarcoidosis patients suggests that these alleles might be protective or associated with a lower risk. Certain alleles appear to be sex-specific, with DQB10603 associated with a higher risk in women and HLA-DRB11101 in men.[15]

In addition to influencing disease susceptibility, HLA genetic markers are also thought to be associated with disease course, severity, and organ involvement. The presence of the HLA-DRB10301 allele is associated with remission and a good prognosis in several ethnic groups (particularly in Europeans and African Americans) and is prevalent in patients with Löfgren syndrome, which has a favorable clinical course.[15,16]

Two studies conducted in Turkey found that the HLA A2, A24, A26, A62, A69, B12, B22, B38, B49, DR4, and DR14 genes were more frequently expressed in sarcoidosis patients, while A24, A26, B62, B7, and DR7 alleles were less frequent and therefore considered to have a protective role.[17,18] Another study found a higher prevalence of HLA DRB115 in patients from the Southeastern Anatolia and Eastern Mediterranean regions of Turkey. The same study also suggested a potential protective role for HLA DRB111 against extrapulmonary sarcoidosis, as it was less frequent in patients with this form of the disease.[19] It has been reported that HLA-DR17 is associated with a non-chronic course, while DR14 and DR15 are associated with chronic disease.[20]

The reported rates of cardiac involvement in sarcoidosis can be as high as 14%.[21] However, there is significant variation in the reported incidence of cardiac sarcoidosis. For example, autopsy studies have reported the prevalence of cardiac involvement to vary between 25% and 58%.[22] The initial findings may present as arrhythmias, heart blocks, and sudden death, depending on the invasion of the heart's conduction system. Myocardial inflammation can lead to dilated cardiomyopathy, and congestive heart failure, localized akinesis, or aneurysms may be observed. Myocardial masses, valvular insufficiencies due to involvement of the papillary muscles, pericarditis, and myocardial ischemia are other rare findings.[23,24]

The decision of treatment in sarcoidosis is made based on clinical and radiological findings and changes in respiratory function test results during follow-up, and there is a need for more useful tools.[25] The presence of life-threatening organ involvement is also of great importance in the initiation and management of treatment.

In this study, the evaluation of HLA polymorphism in patients with sarcoidosis and the genotyping of HLA in sarcoidosis patients with cardiac and other extrapulmonary involvement were investigated to explore the potential for determining the risk of involvement in sarcoidosis patients in advance.

2. Materıals and methods

2.1. Patients

This study included 67 sarcoidosis patients who referred to the Pulmonary Diseases Clinic of Necmettin Erbakan University Medical Faculty Hospital, diagnosed clinically, radiologically, and histopathologically. Exclusion criteria were applied and the control group consisted of 100 bone marrow donors whose HLA typing had been previously performed. Data on patients' pulmonary function tests (PFT), carbon monoxide diffusing capacity (DLCO), posteroanterior (PA) chest x-rays, cardiac MRI reports, echocardiography (ECHO) values, and thoracic CT scans were extracted from hospital information system files.

This research was conducted in accordance with the Helsinki Declaration and good clinical practice guidelines. A detailed explanation of the study protocol was provided and written informed consent was obtained from all participants.

Ethical approval: The study was approved by the local ethics committee (approval no. 2018/1347) and supported by the Scientific Research Projects Unit of our university (project no. 181518023).

2.2. Diagnostic assessment

Diagnostic methods for the patients included transbronchial biopsy, bronchial mucosal sampling, endobronchial ultrasound-guided biopsy (EBUS), mediastinoscopy, skin biopsy, supraclavicular lymph node biopsy, cervical lymph node biopsy, brain biopsy, and a CD4/CD8 ratio in bronchoalveolar lavage (BAL) >3.5, along with clinical and radiological findings. (EBUS biopsy: 32; mediastinoscopy: 15; BAL findings: 9; skin biopsy: 4; bronchial mucosal biopsy: 3; transbronchial biopsy: 1; supraclavicular lymph node biopsy, cervical lymph node biopsy: 1; and brain biopsy: 1).

2.3. Radiological assessment

The Scadding staging system, based on PA chest x-rays, was used for staging sarcoidosis patients. In this classification, stage 0 refers to normal findings on PA chest x-ray, stage 1 to bilateral hilar lymphadenopathy, stage 2 to bilateral hilar lymphadenopathy and parenchymal infiltration, stage 3 to parenchymal infiltration without lymphadenopathy, and stage 4 to parenchymal fibrosis.[26]

Cardiac MRI was performed using a 1.5-Tesla Magnetom Avanto (Siemens, Germany). Spin echo and fat-saturated T2-weighted sequences were used in short-axis, vertical long-axis, and 4-chamber planes. Ten minutes after intravenous administration of 0.1 mmol/kg gadolinium-DTPA (Schering, Germany), short-axis and 4-chamber spin echo and gradient echo sequences were acquired to assess myocardial contrast uptake (8 mm slice thickness, 512 × 512 matrix, 380 mm FOV). Contrast uptake in the left ventricular myocardium was assessed regionally according to the 17-segment model.[27] The examination took 35 to 45 minutes and was interpreted by a single radiologist experienced in cardiac MRI.

2.4. Genetic analysis procedure

Five ml of blood samples were collected in Na-EDTA tubes, centrifuged, and stored at -80°C for batch analysis. After sufficient samples were collected, DNA was isolated using an EZ 1 automated DNA isolation device. HLA gene polymorphism was analyzed, and the differences between patients with and without cardiac MRI findings consistent with sarcoidosis, and the control group, were investigated. Differences between patients with and without other extrapulmonary involvement were also investigated.

HLA typing protocol (SSO-PCR): A 0.2 µL PCR tube was used for each test to be performed per sample. 10 µL DNA was pipetted to the bottom of each PCR tube. Then, for each tube, 15 µL master mix + 24.5 µL dH_2O + 0.5 µL Taq polymerase enzyme mix was added (total 40 µL). The tubes were capped and placed in a thermal cycler, which was run according to the program. At the end of the PCR process, 5 µL of the PCR product was taken and pipetted into a costar plate. Bead mix (Probe mix) from the kit was heated at 55°C for 7 minutes in a heat block. After being sonicated for 15 seconds in a sonicator and vortexed for 15 seconds, 15 µL Bead mix (Probe mix) was pipetted into all wells of the respective test. The plate was sealed with a seal (adhesive tape) and placed in a thermal cycler for hybridization. In the last 5 minutes of the hybridization, 170 µL dilution solution + 0.85 µL streptavidin mix was prepared for each well. At the end of the hybridization, the seal on the plate was removed and 170 µL of the streptavidin mix was pipetted into the wells. The plate was read on a Luminex-Life-Match device and analyzed using the Quick-Type software.

2.5. Exclusion criteria

Pregnant women, patients who declined to participate in the study, patients with malignancies, and patients with uncertain cardiac involvement were excluded.

2.6. Statistical analysis

SPSS version 22 was used for the analyses. "Allele frequency" of HLA alleles was calculated for both patient and control groups, and comparisons were made using the Chi-square or Fisher's exact test. Odds ratio (OR) and confidence interval (CI) were calculated. A P-value < .05 was considered statistically significant.

3. Results

Of the 67 patients in the study group, 48 were female and 19 were male. Pulmonary involvement only was observed in 38 (56.72%) patients. The most frequent extrapulmonary involvement was skin in 16 (23.88%) patients, followed by cardiac in 12 (17.9%), parotid gland in 3 (4.48%), eye in 3 (4.48%), neurological in 2 (2.99%), and rectal involvement in 1 (1.49%) patient. Two patients (2.9%) presented with Löfgren syndrome and 1 (1.49%) with Heerfordt syndrome. Cardiac MRI findings suggestive of sarcoidosis were present in 17.9% of the patients, but no pathological findings were observed on cardiac ECHO.

PFT data were unavailable for 9 patients. Of the remaining 58 patients, 33 (56.9%) had normal FEV1, FVC, and FEV1/FVC values. Mild restrictive lung disease was observed in 12 (20.69%) patients, moderate restrictive lung disease in 5 (8.62%), severe restrictive lung disease in 4 (6.90%), moderate obstructive lung disease in 3 (5.17%), and mixed-type lung disease in 1 (1.72%) patient. Mean PFT values are shown in Table 1.

DLCO measurements were not available for 19 patients, and 2 patients were unable to cooperate with the test. Of the remaining 36 patients, DLCO was normal in 78.26%. Mild restrictive lung disease was present in 6 (13.04%) patients, moderate restrictive lung disease in 2 (4.35%), and severe restrictive lung disease in 2 (4.35%) patients.

Radiological staging was based on chest x-rays. One patient with skin and parotid biopsy proven sarcoidosis declined a chest x-ray, precluding staging. Of the remaining 66 patients, 2 (3.03%) were stage 0, 21 (31.82%) were stage 1, 37 (56.06%) were stage 2, 5 (7.58%) were stage 3, and 1 (1.52%) was stage 4 (Table 2).

Five patients were lost to follow-up. Of the remaining 62 patients, 29 (46.77%) were followed without treatment. Corticosteroids were initiated in 31 (50%) patients. Methotrexate was added to corticosteroid therapy in 3 patients (4.84%) due to corticosteroid side effects or lack of response, and corticosteroids were subsequently tapered and discontinued. Remission was achieved with NSAIDs alone in one Löfgren syndrome patient (1.61%). One patient (1.61%) was followed with non-steroidal anti-inflammatory drugs only. One patient (1.61%) also had a diagnosis of ankylosing spondylitis and was treated with infliximab. Disease progression was observed in one of the 31 patients (3.22%) followed with corticosteroids alone. Remission was achieved in one patient after switching to methotrexate due to corticosteroid side effects. Disease progression occurred in 2 other patients who received methotrexate after corticosteroids, and low-dose corticosteroid therapy was re-initiated. In the patient with ankylosing spondylitis, remission was achieved with infliximab, but due to side effects, the medication was switched to adalimumab by the rheumatology clinic, and disease progression occurred under adalimumab therapy. No progression was observed in the 29 patients followed without treatment, with either complete remission or clinical and radiological stability observed.

There was no statistically significant difference in the expression of HLA-DRB1 alleles between sarcoidosis patients and the control group (Table 3).

There was no statistically significant difference in the expression of HLA-DQB1* and HLA DQA1 alleles between the patient and control groups (Tables 4, 5).

There was no statistically significant difference in the expression of HLA DRB1, HLADQB1, and HLA DQA1 alleles

Table 1

Pulmonary function test results of the patients.

PFT parameter	Mean % ± SD (Min–Max)
FVC	83.03 ± 18.00 (44–116)
FEV1	84.03 ± 19.19 (44–125)
FEF25-75	82.01 ± 29.48 (20–173)
FEV1/FVC	82.75 ± 7.75 (61–97)
DLCO	92.41 ± 22.32 (32–133)
DLCO/VA	96.54 ± 20.20 (30–135)

DLCO = diffusing capacity of the lung for carbon monoxide, DLCO/VA = DLCO/alveolar ventilation ratio, FEF25-75 = forced expiratory flow 25% to 75%, FEV1 = forced expiratory volume in 1 second, FVC = forced vital capacity, Max = maximum, Mean % = percentage mean, Min = minimum, SD = standard deviation.

Table 2

Radiological stages of the patients.

Radiological stage	Number (%)
0	2 (3.03)
1	21 (31.82)
2	37 (56.06)
3	5 (7.58)
4	1 (1.52)
Total	66 (100)

Table 3

Expression of HLA-DRB1 alleles in patients and controls.

HLA DRB1 alleles	Patient group (n = 134) *f* (%)	Control group (n = 200) *f* (%)	*P*	OR (CI)
HLA DRB1*11	26 (19.4)	42 (21.0)	.905	0.905 (0.5241 to1.5649)
HLA DRB1*13	19 (14.1)	19 (9.5)	.189	1.573 (0.7994 to3.0990)
HLA DRB1*04	16 (11.9)	36 (18.0)	.136	0.617 (0.3275 to 1.1652)
HLA DRB1*15	14 (10.4)	16 (8.0)	.444	1.341 (0.6317 to 2.8496)
HLA DRB1*07	13 (9.7)	17 (8.5)	.706	1.156 (0.5421 to 2.4675)
HLA DRB1*03	12 (8.9)	24 (12.0)	.380	0.721 (0.3475 to 1.4974)
HLA DRB1*14	11 (8.2)	15 (7.5)	.812	1.103 (0.4903 to 2.4813)
HLA DRB1*01	6 (4.4)	11 (5.5)	.677	0.805 (0.2905 to 2.2329)
HLA DRB1*16	8 (5.9)	7 (3.5)	.290	1.750 (0.6194 to 4.9475)
HLA DRB1*12	4 (2.9)	4 (2.0)	.566	1.507 (0.3705 to 6.1357)
HLA DRB1*09	3 (2.2)	2 (1.0)	.380	2.238 (0.3690 to 13.5804)
HLA DRB1*10	2 (1.4)	3 (1.5)	.995	0.994 (0.1640 to 6.0357)
HLA DRB1*05	0	1 (0.5)	.667	0.494 (0.0200 to 12.2285)
HLA DRB1*08	0	3 (1.5)	.302	0.209 (0.0107 to 4.0943)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

between sarcoidosis patients with and without cardiac involvement on cardiac MRI. (Tables 6, 7, and 8).

When comparing HLA DQB1 alleles between patients with pulmonary involvement only and those with extrapulmonary involvement, HLA DQB103 and HLA DQB106 were significantly more frequently expressed in patients with pulmonary involvement only ($P = .024$ and $P = .039$). There was no statistically significant difference in the expression of HLA DRB1 alleles. However, when comparing HLA DQA1 alleles, HLA DQA101 was significantly more frequently expressed in patients with extrapulmonary involvement ($P = .003$) (Tables 9, 10, and 11).

4. Discussion

Sarcoidosis is a rare disease with a reported incidence of 5 to 40 cases per 100,000 in Northern European countries.[28] The incidence in Turkey is reported as 4 per 100,000.[4] Sarcoidosis is more common in women. Studies conducted in Turkey have reported a female-to-male ratio between 2.08 and 3.5.[4,19,29] Our study found a similar ratio of 2.5.

Familial sarcoidosis was first reported in 1923 in 2 affected sisters.[5] The most commonly observed familial relationship is between siblings and mother-child pairs.[30] The frequency of familial sarcoidosis in Turkey has been reported between 1% and 3%, and a positive family history has only been demonstrated in first-degree relatives.[4,29]

Previous studies in Turkey have shown that the frequency of extrapulmonary organ involvement in sarcoidosis ranges from 40.6% to 42.9%.[4,31] Similarly, in our study, the frequency of extrapulmonary involvement was 43.2%. The most common extrapulmonary sites of involvement in our study were skin (23.88%), cardiac (17.9%), parotid gland (4.48%), and eye (4.48%).

Staging of sarcoidosis is still based on chest x-ray findings using the Scadding staging system.[32] The majority of our patients had stage 1 or 2 disease, consistent with the ACCESS study findings where stage 3 and 4 sarcoidosis patients represented only 15% of the cases.[33,34] Similarly, in our study, the majority of patients presented in the early stages of the disease, with 31.82% in stage 1 and 56.06% in stage 2. Only 9.1% of patients were in stage 3 or 4, and 3.03% were in stage 0.

Reduced lung volumes, particularly FVC, are common in sarcoidosis patients.[35] FVC is considered the most straightforward and accurate measure for assessing pulmonary function in pulmonary sarcoidosis.[36] Obstructive type impairments are more frequently seen in pulmonary sarcoidosis patients with endobronchial involvement.[35] In our study, the most common pattern of pulmonary function impairment was restrictive, with mild restrictive lung disease observed in 20.6% of patients, moderate restrictive lung disease in 8.6%, and severe restrictive lung disease in 6.9%.

Reviewing our diagnostic methods, EBUS-TBNA has become the most frequently used method in recent years. One study showed no difference in the diagnostic yield of sarcoidosis between conventional bronchoscopic methods and newer methods.[37] However, older, more invasive procedures like mediastinoscopy are now being replaced by EBUS. In our study, the most frequently used diagnostic method was EBUS-TBNA in 32 patients (47.7%), followed by mediastinoscopy (15 patients: 22%) and skin biopsy (4 patients: 5.9%).

The variable presentation of sarcoidosis among different individuals and ethnic groups, as well as its familial occurrence, indicates the significant role of genetics in the etiology and phenotype of this disease. The role of genetics in sarcoidosis is supported by the findings of a Swedish study that included 23,880 sarcoidosis patients between 1964 and 2013. A heritable component was observed in 39% of the analyzed cases, and individuals with ≥1 first-degree relative with sarcoidosis had a 3.7-fold increased risk of developing sarcoidosis.[12]

Studies from various countries have shown that multiple genes may play a role in sarcoidosis and these genes have been linked to the risk of disease development, clinical course, and involvement of specific organs. However, the most frequently reported and strongest genetic association with sarcoidosis is with the HLA gene region, located on the short arm of chromosome 6. Specifically, HLA class II variants, HLA-DRB1 and DQB1, are the most commonly reported and are thought to be associated with sarcoidosis. Individuals carrying the HLA-DRB114:01 allele are more likely to develop chronic disease, while those with HLA-DRB103:01-DRB301:01 alleles tend to develop pulmonary sarcoidosis.[38] Previous studies have shown that sarcoidosis is most commonly associated with HLA-DRB1.[15,16] HLA-DRB103 or 0301 and HLA-DQB102 alleles are frequently found in Löfgren syndrome, which is characterized by a benign course. HLA-DRB114, 12, and 10 alleles are

Table 4

Expression of HLA-DQB1 alleles in patients and controls.

HLA DQB1 Alleles	Patient group (n = 134) *f* (%)	Control group (n = 200) *f* (%)	*P*	OR (CI)
HLA DQB1*02	23 (17.1)	36 (18.0)	.844	0.943 (0.5306 to 1.6792)
HLA DQB1*03	54 (40.3)	88 (44.0)	.502	0.859 (0.5511 to 1.3392)
HLA DQB1*04	1 (0.75)	6 (3.00)	.192	0.243 (0.0289 to 2.0427)
HLA DQB1*05	28 (20.9)	42 (21.0)	.981	0.993 (0.5804 to 1.7015)
HLA DQB1*06	28 (20.9)	28 (14.0)	.100	1.622 (0.9112 to 2.8895)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

Table 5

Expression of HLA-DQA1 alleles in patients and controls.

HLA DQA1 alleles	Patient group (n = 134) *f* (%)	Control group (n = 200) *f* (%)	*P*	OR (CI)
HLA DQA1*01	56 (41.7)	70 (35.0)	.210	1.333 (0.8504 to 2.0906)
HLA DQA1*05	45 (35.5)	73 (36.5)	.584	0.879 (0.5554 to 1.3931)
HLA DQA1*03	19 (14.1)	36 (18.0)	.357	0.752 (0.4111 to 1.3780)
HLA DQA1*02	13 (9.70)	17 (8.50)	.706	1.156 (0.5421 to 2.4675)
HLA DQA1*06	1 (0.75)	1 (0.50)	.776	1.496 (0.0928 to 24.1309)
HLA DQA1*04	0	3 (1.50)	.302	0.209 (0.0107 to 4.0943)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

Table 6

HLA-DRB1 allele expression in sarcoidosis patients with and without cardiac involvement on cardiac MRI.

HLA DRB1 alleles	Cardiac involvement present n:24 *f* (%)	Cardiac involvement absent n:110 *f* (%)	*P*	OR (CI)
HLA DRB1*11	5 (20.83)	21(19.0)	.845	1.115 (0.3735 to 3.3301)
HLA DRB1*03	4 (16.6)	8 (7.27)	.155	2.550 (0.7002 to 9.2860)
HLA DRB1*13	4 (16.6)	15 (13.6)	.700	1.266 (0.3801 to 4.2213)
HLA DRB1*14	4 (16.6)	7 (6.36)	.108	2.942 (0.7873 to 11.0004)
HLA DRB1*07	2 (8.33)	11 (10.0)	.802	0.818 (0.1692 to 3.9560)
HLA DRB1*15	2 (8.33)	12(10.9)	.709	0.742 (0.1550 to 3.5570)
HLA DRB1*01	1 (4.17)	5 (4.55)	.935	0.913 (0.1018 to 8.1907)
HLA DRB1*12	1 (4.17)	3 (2.73)	.709	1.550 (0.1543 to 15.5854)
HLA DRB1*16	1 (4.17)	7 (6.36)	.683	0.639 (0.0750 to 5.4568)
HLADRB1*04	0	16 (14.5)	.139	0.116 (0.0068 to 2.0175)
HLADRB1*09	0	3 (2.73)	.759	0.626 (0.0313 to 12.5342)
HLADRB1*10	0	2 (1.82)	.938	0.885 (0.0412 to 19.0401)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

Table 7

HLA-DQB1 allele expression in sarcoidosis patients with and without cardiac involvement on cardiac MRI.

HLA DQB1 alleles	Cardiac involvement present n:24 *f* (%)	Cardiac involvement absent n:110 *f* (%)	*P*	OR(CI)
HLA DQB1*03	7 (29.1)	47 (42.7)	.223	0.551 (0.2118 to 1.4384)
HLA DQB1*05	6 (25.0)	22 (20.0)	.586	1.333 (0.4735 to 3.7548)
HLA DQB1*02	6 (25.0)	17 (15.4)	.266	1.823 (0.6326 to 5.2569)
HLA DQB1*06	5 (20.8)	23 (20.9)	.993	0.995 (0.3357 to 2.9520)
HLA DQB1*04	0	1 (0.91)	.808	1.489 (0.0589 to 37.6805)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

Table 8

HLA-DQA1 allele expression in sarcoidosis patients with and without cardiac involvement on cardiac MRI.

HLA DQA1 alleles	Cardiac involvement present n:24 *f* (%)	Cardiac involvement absent n:110 *f* (%)	*P*	OR (CI)
HLA DQA1*01	11 (45.8)	45 (40.9)	.658	1.222 (0.5027 to 2.9714)
HLA DQA1*05	11 (45.8)	34 (30.9)	.164	1.891 (0.7697 to 4.6477)
HLA DQA1*02	2 (8.33)	11 (10.0)	.802	0.818 (0.1692 to 3.9560)
HLA DQA1*03	0	19 (17.2)	.105	0.095 (0.0056 to 1.6429)
HLA DQA1*06	0	1 (0.91)	.808	1.489 (0.0589 to 37.6805)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

Table 9

HLA-DRB1 allele expression in patients with extrapulmonary involvement vs. pulmonary sarcoidosis only.

HLA DRB1 alleles	Extrapulmonary involvement present (n:58) *f* (%)	Pulmonary sarcoidosis only (n:76) *f* (%)	*P*	OR(CI)
HLA DRB1*13	11 (18.9)	8(10.5)	.170	1.989 (0.7439 to 5.3202)
HLA DRB1*11	9 (15.5)	17 (22.3)	.322	0.637 (0.2612 to 1.5560)
HLA DRB1*15	8 (13.7)	6 (17.89)	.274	1.866 (0.6097 to 5.7154)
HLA DRB1*03	5 (8.62)	7 (9.21)	.905	0.929 (0.2795 to 3.0942)
HLA DRB1*04	5 (8.62)	11 (14.4)	.305	0.557 (0.1823 to 1.7045)
HLA DRB1*07	5 (8.62)	8(10.5)	.712	0.801 (0.2480 to 2.5932)
HLA DRB1*14	4 (6.90)	7 (9.21)	.629	0.730 (0.2032 to 2.6237)
HLA DRB1*16	4 (6.90)	4 (5.26)	.693	1.333 (0.3190 to 5.5724)
HLA DRB1*01	3 (5.17)	3 (3.95)	.734	1.327 (0.2579 to 6.8297)
HLA DRB1*12	3(5.17)	1 (1.32)	.227	4.090 (0.4144 to 40.3896)
HLA DRB1*10	1(1.72)	1 (1.32)	.847	1.315 (0.0806 to 21.4905)
HLA DRB1*09	0	3 (3.95)	.259	0.179 (0.0091 to 3.5446)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

observed in British, German, Dutch, and Japanese populations, while HLA-DRB11501 is observed in Finnish populations, suggesting a risk for sarcoidosis development. In Americans, expression of HLA-DRB11101 is associated with a high risk in both Caucasian and African American populations.[10] In our study, there was no statistically significant difference in the expression of HLA-DRB1, HLA DQB1, and HLA DQA1 alleles between sarcoidosis patients and the control group.

A literature review revealed that certain genetic variants (HLA-DRB1 and HLA-DQB10601) are associated with the development of cardiac sarcoidosis. This has important implications for the screening and management of patients with systemic sarcoidosis, as identifying these variants can aid in risk stratification for cardiac sarcoidosis development. Moreover, it may facilitate earlier use of advanced cardiovascular imaging to support diagnosis and guide personalized treatment. Importantly, cardiac sarcoidosis can present as sudden cardiac death. Therefore, identification of positive genetic variants may lead to a lower threshold for evaluating arrhythmias and considering the use of an implantable cardioverter defibrillator.[39,40] In our study, HLA-DRB1, DQB1, and DQA1 typing and allele identification were performed in sarcoidosis patients, and statistical analysis was conducted by comparing groups based on organ involvement. A total of 25 alleles were identified in both the patient and control groups. There was no statistically significant difference in the expression of HLA DRB1, HLADQB1, and HLA DQA1 alleles between sarcoidosis patients with and without cardiac involvement on cardiac MRI.

In addition to indicating the risk of developing disease, HLA genetic markers are also considered to be associated with the course, severity, and organ involvement of the disease. The presence of the HLA-DRB10301 allele is associated with remission and good prognosis in many ethnic groups (especially in European and African Americans) and has high prevalence in patients with Löfgren syndrome, which has a favorable clinical course.[15,16]

Two studies conducted in Turkey found that HLA A2, A24, A26, A62, A69, B12, B22, B38, B49, DR4, and DR14 genes are more frequently expressed in sarcoidosis patients, while A24, A26, B62, B7, and DR7 alleles are less frequent and are therefore considered protective.[17,18] Another study found a higher prevalence of HLA DRB115 in patients from the Southeastern Anatolia and Eastern Mediterranean regions of Turkey. The same study suggested a potential protective role for HLA DRB111 against extrapulmonary sarcoidosis due to its lower frequency in such patients.[19] In our study, HLA DQB103 and HLA DQB106 alleles were significantly more frequently expressed in patients with only pulmonary involvement than those with extrapulmonary involvement (P = .024 and P = .039). HLA DQA101 was significantly more frequently expressed in patients with extrapulmonary involvement (P = .003). HLA DRB111 allele prevalence was 15.5% in the extrapulmonary sarcoidosis group and 22.37% in the pulmonary sarcoidosis only group, but this difference was not statistically significant.

5. Limitations

Cardiac MRI was used to assess cardiac involvement in our study. The definitive diagnosis of cardiac sarcoidosis requires

Table 10

HLA-DQB1 allele expression in patients with extrapulmonary involvement vs. pulmonary sarcoidosis only.

HLA DQB1 alleles	Extrapulmonary involvement present (n:58) *f* (%)	Pulmonary sarcoidosis only (n:76)	*P*	OR (CI)
HLA DQB1*03	17 (29.3)	37 (48.6)	.0247	0.437 (0.2122 to 0.9001)
HLA DQB1*06	17 (29.3)	11 (14.4)	.0395	2.450 (1.0438 to 5.7510)
HLA DQB1*05	13 (22.4)	15 (19.7)	.7058	1.174 (0.5089 to 2.7122)
HLA DQB1*02	10 (17.2)	13 (17.1)	.9835	1.009 (0.4081 to 2.4980)
HLA DQB1*04	1 (1.72)	0	.3993	3.991 (0.1596 to 99.7847)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

Table 11

HLA-DQA1 allele expression in patients with extrapulmonary involvement vs. pulmonary sarcoidosis only.

HLA DQA1 alleles	Extrapulmonary involvement present (n:58) *f* (%)	Pulmonary sarcoidosis only (n:76)	*P*	OR(CI)
HLA DQA1*01	30 (51.7)	26 (34.2)	.0030	3 (1.4527 **to** 6.1955)
HLA DQA1*05	17 (29.3)	28 (36.8)	.3612	0.710 (0.3416 **to** 1.4790)
HLA DQA1*02	5 (8.62)	8 (10.5)	.7123	0.801 (0.2480 **to** 2.5932)
HLA DQA1*03	5 (8.62)	14 (18.4)	.1149	0.417 (0.1412 to 1.2364)
HLA DQA1*06	1 (1.72)	0	.6075	0.430 (0.0172 to 10.7544)

CI = confidence interval, *f* = frequency, n = number of alleles, OR = odds ratio, *P* = *P*-value.

endomyocardial biopsy (EMB), which is often difficult to obtain. Previous studies have reported a success rate of only about 25%.[41] Furthermore, granulomas are rare in cardiac sarcoidosis, and voltage-guided biopsy has only increased the success rate to approximately 50% with technological advancements.[42] Recently, combined MRI/PET has been proposed for diagnosis. However, limitations of FDG-PET include FDG uptake in non-inflamed myocardium and the potential for atrial fibrillation or conduction abnormalities to affect regional glucose utilization. There are also risks associated with radiation exposure.[43] Therefore, advanced imaging was not performed to diagnose cardiac sarcoidosis.

The lack of statistically significant differences in the expression of HLA DRB1, HLADQB1, and HLA DQA1 alleles between sarcoidosis patients with and without cardiac involvement on cardiac MRI in our study might be attributed to the single-center design and the relatively small sample size. Further multicenter studies with larger sample sizes are needed in Turkey and globally to investigate this further.

6. Conclusion

Cardiac MRI findings suggestive of sarcoidosis were present in 17.9% of our patients, but there were no pathological findings on echocardiography. This suggests that cardiac echocardiography alone may not be sufficient to evaluate cardiac involvement in sarcoidosis. Skin involvement was the most frequent extrapulmonary finding, occurring in 23.8% of patients. HLA DQB103 and HLA DQB106 were significantly more frequently expressed in patients with only pulmonary involvement and may have a protective role against extrapulmonary organ involvement. HLA DQA101 was more frequently expressed in patients with extrapulmonary involvement and could be a risk factor. There was no statistically significant difference in the expression of HLA DRB1, HLADQB1, and HLA DQA1 alleles between patients with and without cardiac involvement on cardiac MRI. Further studies are needed to evaluate HLA genotyping in cardiac sarcoidosis patients in Turkey.

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