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ORIGINAL ARTICLE

The relationship between COVID-19 and HLA in kidney transplant recipients, an evaluation of predictive and prognostic factors

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Abstract

Introduction: The purpose of this study was to determine the predictive and prognostic factors for COVID-19 infection and its relationship with human leukocyte antigen (HLA) in kidney transplant recipients.

Material and method: Three hundred fifty kidney transplant recipients were included in the study. Recipients were divided into two groups: COVID-19(+) (n = 100) and control (n = 250). The relationships between HLA frequencies, COVID-19 infection, and prognostic factors (age, donor type, immunosuppression protocol, etc.) were then evaluated. Logistic regression analysis, heatmap, and decision tree methods were used to determine predictive and prognostic factors. The study was performed retrospectively.

Results: Advanced age and deceased transplantation emerged as predictive of SARS-CoV-2 infection, while the presence of HLA-A*11, the HLA match ratio, and high-dose tacrolimus were identified as prognostic factors in kidney transplant recipients. HLA-A10, HLA-B*13, HLA-B22, and HLA-B*55 were shown to be associated with SARS-CoV-2 infection at univariate analysis, and HLA-B*57, HLA-DRB1*11, and HLA-DRB1*13 at logistic regression analysis.

Conclusion: HLA-A10, HLA-B*13, HLA-B*55, HLA-B*57, HLA-DRB1*11, and HLA-DRB1*13 were identified for the first time in the literature associated with SARS-CoV-2 infection in kidney transplant recipients.

KEYWORDS
human leukocyte antigen, renal transplantation, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by the RNA virus Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has recently resulted in a global pandemic.[1] SARS-CoV-2 spreads rapidly, endangering both global health and the world economy, and is one of the leading international causes of death. Diagnosing positive cases, providing emergency care for individuals affected by COVID-19, and preventing further infection in the population are essential. It is therefore of the utmost importance to identify the genetic, epigenetic, and environmental factors that lead to susceptibility to COVID-19.[2] We report susceptibility to COVID-19 among

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kidney transplant recipients based on genetic, clinical, and demographic factors.

The continuing COVID-19 pandemic has had a significant impact on solid organ transplantation (SOT) worldwide and has become a threat to the lives of SOT recipients, as to other members of the community.[3] Since emerging as a specialty field in the 1980s, SOT has progressed rapidly due to advances in surgical techniques, immunosuppression, and genetics. The number of transplant operations performed and the number of SOT transplant recipients in society have therefore both increased.[4,5] As the number of cases increases, the probability of encountering the SARS-CoV-2 virus also rises. Organ transplant recipients are thought to be more susceptible to COVID-19 infection due to the immunosuppressive medications they use. This makes it even more important to investigate the genetic and environmental factors that predispose to COVID-19 infection. One of the most important issues requiring investigation is human leukocyte antigens (HLAs), which are necessary for the immune system to distinguish between self and non-self (foreign) cells, and which are associated with family and population studies, transplantation, infectious diseases, autoimmune diseases, and many types of cancer.[6]

The first study of the relationship between SARS and HLA was performed in 2003.[7] HLA-B*46:01 was found to be related to SARS disease. With the emergence of the SARS-CoV-2 virus, studies investigating the relationship between viral infection and HLA have acquired renewed importance.[8–10] Although common, possibly related HLAs have been described in studies from several countries, regional differences have also been observed.[11–13] Due to the regional variation in the frequency of HLA types, studies investigating HLAs and their relationship with COVID-19 in different regions are essential.

The investigation of susceptibility factors for SARS-CoV-2 infection in organ transplant recipients is critical since these have to use immunosuppression agents throughout their lives. The relationship between COVID-19 and HLAs, which play a critical role in the immunological response against RNA viruses as well as in SOTs (especially kidney transplants), has previously been investigated.[14] The aim of the present study was to investigate the HLA type of the renal transplant recipient, donor-recipient tissue compatibility, and the effects of these genetic factors on COVID-19.

2 | MATERIALS AND METHODS

2.1 | Sample size

In the study, clinical significance was accepted when the difference between the incidence of certain HLAs in kidney transplant recipients with and without COVID-19 diagnosis was at least 10%. Under these conditions ($p1 = 0.15$; $p2 = 0.05$), assuming 80% power, .05 margin of error and allocation ratio (N2 / N1) ratio is 2.5, the minimum number of samples to be included in the study according to the two-ratio difference test is group 1 (COVID-19(+)) for n = 90 and for group 2 (COVID-19(-)) n = 224. The sample number estimation of the study was made by using G*Power 3.1.9.2 program.[15]

One hundred kidney transplant recipients with COVID-19 diagnosis and 250 kidney transplant recipients without COVID-19 diagnosis were included in the study in order to obtain the minimum number of volunteers against the loss in follow-up and the possibility of not having access to patient data. Despite this, the data of all patients included in the project were accessed; has been included in the analysis.

2.2 | Subjects

One hundred kidney transplant recipients diagnosed with COVID-19 by means of PCR tests and presenting to our center between April 2020 and February 2021 were included in the study. Patients who underwent kidney transplantation in our center, all immunological tests including HLA Ags were performed in our center, the diagnosis and treatment process of COVID-19 was performed in our hospital, all infections, except for COVID-19 infection, were excluded at the time of diagnosis, no active rejection attack, and all data were reliably accessed was included in the study. In addition, we included 250 kidney transplant recipients with these characteristics and similar demographic characteristics, who applied to our center during the same period, as a control group. This study was approved by the local Clinical Research Ethics Committee (28.04.2021/KAEK-219). The study was conducted in our center's HLA Tissue Typing Laboratory, which is accredited by the Turkish Ministry of Health. External quality control tests of the European Immunogenetics Federation (EFI) and the UK National External Quality Assessment Service (NEQAS) are routinely applied in our laboratory.

2.3 | Molecular analysis of HLA class I and class II alleles

Genomic DNA of healthy controls and patients infected with SARS-CoV-2 were isolated from 200 ul peripheral blood samples using a Bio-robot EZ1 advanced XL magnetic bead-based workstation (Qiagen, Germany). HLA-A, HLA-B, and HLA-DRB1 genotyping were performed on all subjects by the low-resolution polymerase chain reaction with sequence-specific oligonucleotide probe (PCR-eRES,SSO) hybridization method using Luminex technology (IMMUCOR-Lifecodes, Georgia).

2.4 | Serological typing of HLA class I and class II alleles

Whole blood was collected on citrate phosphate dextrose, and lymphocytes were isolated by centrifugation on Ficoll-Hypaque. The isolated cells were counted and adjusted to 3×10^6 cells/mL. Serological typing was performed on lymphocyte suspensions using the microlymphocytotoxicity technique (standard NIH) and local set of sera. Data for two HLA alleles were analyzed as serological typing, HLA-A10 and HLA-B22.

2.5 | Statistical analysis

Descriptive statistics are presented as frequency, percentage, mean, standard deviation, median, minimum, maximum, 25%-75% percentile (Q1-Q3), or IQR values. Normality assumptions were checked by examining the histogram, q-q plots, skewness, and kurtosis values with the Shapiro Wilk test.

The independent two-sample *t* test was used to analyze the difference in numerical data between the two groups when the data conformed to normal distribution, and the Mann-Whitney *U* test was used when the data were not normally distributed.

The Pearson Chi-Square test was used to evaluate relationships between categorical data when the expected value was less than 5 and the ratio of cells was less than 20%, while Fisher's exact test was used when the ratio of cells exceeded 20%. Bonferroni correction was made in pairwise comparisons for the results found to be significant in the multiway tables.

Variables with a p value less than 0.20 (using the Chi-square test with categorical variables and the Mann-Whitney *U* test with continuous variables) were included in the binary logistic regression analysis to identify risk factors for COVID-19(+). Covariates $p < 0.5$ were selected from those that were statistically in the logistic regression analysis. Variance inflation factors (VIFs) were employed to provide a standardized multicollinearity measure for estimating the risk of bias in multivariate models.[16] Independent variables were analyzed for multicollinearity (VIF > 3), and since the VIF value exceeded 3 when the TOTAL MATCH variable was included in the model, this variable was excluded from the binary logistic regression model. In addition, the TYPE OF DONOR variable was excluded from the model since it is a highly dominant variable in predicting COVID-19(+) and affects all other parameters. p values less than 0.05 were considered statistically significant.

2.6 | Data visualization

In addition to the classical statistical analyses, we applied nonlinear techniques to cluster, classify, and visualize data to provide insights. We used *heatmap* and *rpart* decision tree libraries from the R package [R Core Team (2017)]. *CART* (Classification and Regression Trees) algorithm is a powerful supervised learning tool to split data attributes into sub-groups based on an error measure such as sum of squared errors. R implementation of this algorithm *RPART* (Recursive Partitioning And Regression Trees) is used to produce visually meaningful tree plots. The resulting decision trees can be manipulated by three parameters in the *rpart* function namely *cp* (complexity parameter), *minbucket* (minimum number samples in leaves), and *minsplit* (minimum number of observations in a node to be split). We used only the statistically significant variables from the univariate analysis in forming the heatmap and the decision tree. For the heatmap, we first formed a variable-to-variable similarity matrix for the selected variables. We then counted the number of COVID-19(+) patients that are similar (in the same category) for each variable pair in the matrix and divided the count by the total COVID-19(+). This made sure that the minimum value in the matrix is 0 and the maximum is 1. Finally, we used the *heatmap* (with dendogram clustering) function on the similarity matrix, with a five-color scale (white, grey, pink, red, and magenta), each color showing 0–20%, 20–40%, 40–60%, 60–80%, and 80–100% similarity respectively, among all COVID-19(+) patients in terms of pairs of variables in the similarity matrix. Naturally, all the elements in the diagonal of the similarity matrix are magenta (100%).

3 | RESULTS

3.1 | Patient management

According to the Clinical Progression Scale published by the World Health Organization COVID-19 working group in August 2020, the clinical manifestation of COVID-19 infection is examined under five headings[17]: uninfected, ambulatory mild disease, hospitalized with moderate disease, hospitalized with severe disease, and dead. According to this report, patients with a score of 4 or 5 should be hospitalized. A score of 4 is given if the patient needs supportive treatment without oxygen therapy, and a score of 5 if the patient also needs oxygen therapy. Scores of 6 or more mean that the patient requires intubation and mechanical ventilation, and treatment must be continued under intensive care conditions.

It was determined that 25 patients (25%) were hospitalized and two of these patients were taken to the intensive care unit. Antiproliferative (MMF, CellCept) drugs were discontinued in COVID-19(+) kidney transplant recipients. Maintenance immunosuppression treatment was continued with 5 mg prednisolone and tacrolimus. Tacrolimus was also discontinued in two patients who needed intensive care, while the steroid dose was increased to 20 mg. Patient loss due to COVID-19 infection was not observed.

3.2 | Demographics, clinical characteristics, and statistical results

Presence of SARS-CoV-2 infection, etiology of end-stage renal disease, postoperative periods after transplantation, drugs used in the transplant process, and blood tacrolimus levels are shown in Table 1. The COVID-19(+) patients (median age: 49.5; IQR:37.5-58) were statistically significantly older than the control group (median age: 40; IQR:31-56) ($p = 0.007$ No statistically significant difference was found between the gender distributions of COVID-19(+) and COVID-19(-) patients, median post-op times after kidney transplantation, or renal failure etiologies ($p = 0.548$, $p = 0.934$, $p = 0.425$). When examined in terms of donor type, the deceased donor rate was 21% in patients with COVID-19 infection compared to 5.6% in those without COVID-19 infection, the difference being statistically significant ($p < 0.001$). When these two groups (living or deceased donor) were compared in terms of the study data, a statistically significant difference was observed

TABLE 1 Patients' demographic and clinical characteristics and their relationship with SARS-CoV-2 infection

A														
		COVID19 DISEASE STATUS												
		COVID19 (+) (n = 100)						***CONTROL (n = 250)***						
		n	**Mean**	**SD**	**Median**	**Q1**	**Q3**	**n**	**Mean**	**SD**	**Median**	**Q1**	**Q3**	***p***
Age		**100**	**47.41**	**13.40**	**49.5**	**37.5**	**58**	**250**	**43.83**	**14.74**	**40**	**31**	**56**	**0.007_t**
Gender (n,%)	Male	74 (74%)	–	–	–	–	–	177 (70.8%)	–	–	–	–	–	0.548_a
	Female	26 (26%)	–	–	–	–	–	73 (19.2%)	–	–	–	–	–	
Time After Transplantation (month)		100	55.81	39.74	44.29	23.69	81.51	250	55.08	41.65	49.81	21.85	87.75	0.934_b
Etiology of CKD (n,%)	Hypertensive Nephropathy	13 (13%)	–	–	–	–	–	50 (20%)	–	–	–	–	–	0.425_a
	Diabetic Nephropathy	11 (11%)	–	–	–	–	–	23 (9.2%)	–	–	–	–	–	
	Chronic Glomerulonephritis	19 (19%)	–	–	–	–	–	34 (13.6%)	–	–	–	–	–	
	Nephrolithiasis	7 (7%)	–	–	–	–	–	24 (9.6%)	–	–	–	–	–	
	Focal Segmental Glomerulosclerosis	1 (1%)	–	–	–	–	–	8 (3.2%)	–	–	–	–	–	
	Vesicoureteral Reflux	6 (6%)	–	–	–	–	–	8 (3.2%)	–	–	–	–	–	
	Familial Mediterranean Fever	1 (1%)	–	–	–	–	–	6 (2.4%)	–	–	–	–	–	
	Other	22 (22%)	–	–	–	–	–	56 (22.4%)	–	–	–	–	–	
	Unkown	20 (20%)	–	–	–	–	–	41(16.4%)	–	–	–	–	–	
Type of Donor (n,%)	**Living**	**79 (79%)**	–	–	–	–	–	**236 (94.4%)**	–	–	–	–	–	**$<0.001_a$**
	Deceased	**21 (21%)**	–	–	–	–	–	**14 (5.6%)**	–	–	–	–	–	
Induction Theraphy (n,%)	ATG	46 (46%)	–	–	–	–	–	105 (42%)	–	–	–	–	–	0.495_a
	Basiliximab	44 (44%)	–	–	–	–	–	126 (50.4%)	–	–	–	–	–	0.279_a
Maintenance Theraphy (n,%)	Triple Treatment	98 (98%)	–	–	–	–	–	238 (95.2%)	–	–	–	–	–	0.365_a
	CsA	7 (7%)	–	–	–	–	–	17 (6.8%)	–	–	–	–	–	0.947_a
	mTOR inhibitors	8 (8%)	–	–	–	–	–	25 (10%)	–	–	–	–	–	0.563_a
History of Rejection Attack (n,%)	Yes	15 (15%)	–	–	–	–	–	53 (21.2%)	–	–	–	–	–	0.185_a
	No	85 (85%)	–	–	–	–	–	197 (78.8%)	–	–	–	–	–	
Blood Tacrolimus Level (ng/mL)		100	6.93	2.5	6.45	5.2	8.1	250	6.75	3.45	6.2	4.5	8.1	0.207_b

(Continues)

TABLE 1 (Continued)

B														
		HOSPITALIZATION STATUS FOR COVID19(+)												
		HOSPITALIZATION (+) (n = 25)						***HOSPITALIZATION (n = 75)***						
		n	**Mean**	**SD**	**Median**	**Q1**	**Q3**	**n**	**Mean**	**SD**	**Median**	**Q1**	**Q3**	***p***
Age		25	46.96	13.00	49.00	36.00	55.00	75	47.56	13.61	50.00	39.00	59.00	0.847_t
Gender (n,%)	Male	18 (72%)	–	–	–	–	–	56 (74.7%)	–	–	–	–	–	0.792_a
	Female	7 (28%)	–	–	–	–	–	19 (25.3%)	–	–	–	–	–	
Time After Transplantation (month)		25	53.98	43.54	32.30	22.21	80.36	75	56.42	38.68	44.75	27.70	82.66	0.566_b
Etiology of CKD (n,%)	Hypertensive Nephropathy	4 (16%)	–	–	–	–	–	9 (12%)	–	–	–	–	–	0.493_a
	Diabetic Nephropathy	2 (8%)	–	–	–	–	–	9 (12%)	–	–	–	–	–	
	Chronic Glomerulonephritis	2 (8%)	–	–	–	–	–	17 (22.7%)	–	–	–	–	–	
	Nephrolithiasis	2 (8%)	–	–	–	–	–	5 (6.7%)	–	–	–	–	–	
	Focal Segmental Glomerulosclerosis	0 (0%)	–	–	–	–	–	1 (1.3%)	–	–	–	–	–	
	Vesicoureteral Reflux	3 (12%)	–	–	–	–	–	3 (4%)	–	–	–	–	–	
	Familial Mediterranean Fever	0 (0%)	–	–	–	–	–	1 (1.3%)	–	–	–	–	–	
	Other	8 (32%)	–	–	–	–	–	14 (18.7%)	–	–	–	–	–	
	Unkown	4 (16%)	–	–	–	–	–	16 (21.3%)	–	–	–	–	–	
Type of Donor (n,%)	**Living**	**16 (64%)**	**–**	**–**	**–**	**–**	**–**	**63 (84%)**	**–**	**–**	**–**	**–**	**–**	**0.033_a**
	Deceased	**9 (36%)**	**–**	**–**	**–**	**–**	**–**	**12 (16%)**	**–**	**–**	**–**	**–**	**–**	
Induction Theraphy (n,%)	ATG	15 (60%)	–	–	–	–	–	31 (41.3%)	–	–	–	–	–	0.105_a
	Basiliximab	8 (32%)	–	–	–	–	–	36 (48%)	–	–	–	–	–	0.163_a
Maintenance Theraphy (n,%)	Triple Treatment	24 (96%)	–	–	–	–	–	74 (98.7%)	–	–	–	–	–	0.439_a
	CsA	3 (12%)	–	–	–	–	–	4 (5.3%)	–	–	–	–	–	0.362_a
	mTOR inhibitors	2 (8%)	–	–	–	–	–	6 (8%)	–	–	–	–	–	0.999_a
History of Rejection Attack (n,%)	Yes	5 (20%)	–	–	–	–	–	10 (13.3%)	–	–	–	–	–	0.518_a
	No	20 (80%)	–	–	–	–	–	65 (86.7%)	–	–	–	–	–	
Blood Tacrolimus Level (ng/mL)		**25**	**7.78**	**2.73**	**7.70**	**6.30**	**10.0**	**75**	**6.58**	**2.53**	**6.2**	**4.80**	**7.50**	**0.01_b**

(a) Pearson CS, (b) MWU, (t) *t* tests.

between age and HLA matches (Supplementary Information 1). No statistically significant difference was observed in parameters other than these criteria.

No statistically significant difference was found between the distributions of treatments used by COVID-19(+) and COVID-19(-) patients during the transplant process, rates of rejection attacks, or blood tacrolimus levels. When examined in terms of rejection types, 86.66% of rejections in the COVID-19(+) group appeared as T-cell mediated rejection. Similarly, it was observed that 86.79% of the rejections in the COVID-19(+) group were T-cell mediated rejection, and there was no statistical significance between the groups. There is no significant difference between the two groups in the other two rejection types (AMR and mix type). While AMR and mixed type rejection rates were similar in the COVID-19(+) group (6.60%); and the AMR rate was 7.54% and the mixed type rejection rate was 5.66% in the control group. The blood tacrolimus level (ng/mL) of the hospitalization (+) patients (median: 7.70; IQR: 6.3-10.0) was statistically significantly higher than that in the hospitalization (-) group (median:6.2; IQR:4.8-7.5) ($p = 0.01$). A significant difference was observed between the distributions of donor type ($p = 0.033$). No statistically significant difference was found between the other parameters of the hospitalization groups.

Kidney transplant recipients were compared according to each HLA type in terms of presence of COVID-19 and hospitalization (Table 2). A statistically significant relationship was found between COVID-19 status and HLA-A10 ($p = 0.007$), HLA-B*13 ($p = 0.006$), HLA-B22 ($p = 0.023$), and HLA-B*55 ($p = 0.042$). However, the presence of the HLA-B*13 allele increased the probability of occurrence of COVID-19 infection 2.63-fold (95% CI 1.289-5.369). The presence of the HLA-B*55 allele increased the probability of COVID-19 occurrence 2.94-fold (95% CI 1.050-8.203). The only significant allele in terms of hospitalization was HLA-A*11 ($p = 0.030$), the presence of which increased the probability of hospitalization 3.38-fold (95% CI 1.197-9.553). No statistical significance was observed between whether these alleles were homozygous or heterozygous (Supplementary Information 2).

A comparison of patients with or without SARS-CoV-2 infection and those who were hospitalized or not hospitalized in terms of HLA-A, HLA-B, HLA-DRB1, and total match values are shown in Table 3. A statistically significant relationship was found between HLA match and presence of COVID-19 infection for all HLAs ($p < 0.001$). In pairwise comparisons using the Bonferroni correction, the mismatch rate for all alleles was higher among the COVID-19 patients than in the control group, while the match ratio to one allele was higher in the control group than in the COVID-19 patients. No difference in the proportions of patients with two alleles matched was observed between the COVID-19 and control groups. When examining how many of the six alleles had a match, full-mismatch and 1/6 match were higher in the COVID-19(+) group, while 3/6 match was higher in the control group. In addition, 2/6, 4/6, 5/6, and 6/6 matches exhibited no difference between the two groups. No statistically significant relationship was determined between compliance variables and hospitalization for all HLAs.

3.3 | Logistic regression model

Multivariate analysis was applied using logistic regression after adjusting for age and blood tacrolimus levels in order to examine which HLA alleles are associated with COVID-19(+) (Table 4). The analysis showed that presence of the HLA-B*57 allele was independently correlated with COVID-19(+) and a 3.58-fold increase in the probability of COVID-19(+) (OR = 3.58, 95%CI = 1.10–11.64, $p = 0.031$). HLA-DRB1*11 was also shown to be independently associated with COVID-19(+) (OR = .60, 95%CI = .37–.97, $p = 0.042$) and a 40% decrease in the probability of COVID-19(+). HLA-DRB1*13 also emerged as independently associated with COVID-19(+) (OR = .43, 95%CI = .19–.90, $p = 0.033$) and a 57% decrease in the probability of COVID-19(+). A one HLA-A allele match compared to N/A match resulted in a 60% decrease in the probability of COVID-19(+) (OR = .40, 95%CI = .24–.66, $p < 0.001$). Two HLA-A allele matches compared to N/A match resulted in a 61% decrease in the probability of COVID-19(+) (OR = .39, 95%CI = .18–.83, $p = 0.015$). A one HLA-B allele match compared to N/A match produced a 77% decrease in the probability of COVID-19(+) (OR = .23, 95%CI = .14–.37, $p < 0.001$). Two HLA-B allele matches compared to N/A match resulted in a 69% decrease in the probability of Covid-19(+) (OR = .31, 95%CI = .14–.65, $p = 0.002$). A one HLA-DRB1 allele match compared to N/A match caused an 80% decrease in the probability of COVID-19(+) (OR = .20, 95%CI = .11–.37, $p < 0.001$). A two HLA-DRB1 allele match compared to N/A match resulted in a 72% decrease in the probability of COVID-19(+) (OR = .28, 95%CI = .13–.59, $p = 0.001$). The covariates of age and blood tacrolimus levels were not independently associated with COVID-19(+). However, blood tacrolimus level, HLA-B*13, HLA-B*18, and HLA-B*35 were insignificant. HLA-B*44, HLA-B*55, and HLA-DRB1*15 were not independently associated risk factors for COVID-19(+).

3.4 | Similarity heatmap

The heatmap in Figure 1 shows that the HLA-A match and HLA-B match rates are 60–80% similar in COVID-19(+) patients. The similarity rate in terms of whether HLA-DRB1*11 is found with the HLA-A10 allele is 80–100%. In addition, the attribute with the highest similarity to other attributes is HLA-DRB1*11. HLA-DRB1*11 has a similarity ratio of 60–80% with the parameters HLA-DRB1*15, HLA-B*13, HLA-DRB1*13, HLA-B*57, and HLA-B22. In the heatmap, COVID-19 Disease Status, Age, Blood Tacrolimus Level, Type of Donor, HLA-A10, HLA-B*13, HLA-B22, HLA-B*55, HLA-B*57, HLA-DRB1*11, HLA-DRB1*13, HLA-DRB1*15 have two categories, HLA-A MATCH, HLA-B MATCH, HLA-DRB1 MATCH variables have three categories, and TOTAL HLA MATCH has a total of seven categories. It is also worth noting that the similarity rate between two binary categories will be high when both categories have few positive occurrences because the absence of both attributes amount to similarity. This caveat must be considered while using similarity measures with categoric data.

TABLE 2 Comparison of HLA allele frequencies between the COVID-19 patient group and the control group among kidney transplant recipients

A								
	COVID19 DISEASE STATUS				HOSPITALIZATION STATUS FOR COVID19			
	COVID19 + (2n = 200)	Control (2n = 500)	Statistical analysis		Hosp (+) (2n = 50)	Hosp (-) (2n = 150)	Statistical analysis	
	n - AF(%)	n - AF (%)	OR - (95%CI)	*p*	n - AF(%)	n - AF (%)	OR - (95%CI)	*p*
HLA-A*01	21 (10.50)	57 (11.40)	.912 (.537-1.548)	0.732_a	2 (4.00)	19 (12.47)	.29 (.064-1.280)	0.083_a
HLA-A*02	42 (21.00)	98 (19.60)	1.090 (.727-1.636)	0.676_a	7 (14.00)	35 (23.30)	.53 (.221-1.295)	0.161_a
HLA-A*03	23 (11.50)	67 (13.40)	.840 (.507-1.391)	0.497_a	9 (18.00)	14 (9.30)	2.13 (.861-5.283)	0.096_a
HLA-A*04	0 (.00)	6 (1.2)	–	0.191_b	0 (.00)	0 (.00)	–	–
HLA-A10$_s$	**4 (2.00)**	**0 (.00)**	–	**0.007_a**	0 (.00)	4 (2.7)	–	0.574_b
HLA-A*11	16 (8.00)	39 (7.80)	1.03 (.560-1.885)	0.929_a	**8 (16.00)**	**8 (5.30)**	**3.38 (1.197-9.553)**	**0.030_b**
HLA-A*23	9 (4.50)	21 (4.20)	1.75 (.484-2.389)	0.859_a	1 (2.00)	8 (5.3)	.36 (.044-2.970)	0.455_b
HLA-A*24	30 (15.00)	77 (15.40)	.97 (.613-1.532)	0.894_a	8 (16.00)	22 (14.70)	1.11 (.459-2.675)	0.819_a
HLA-A*25	2 (1.00)	5 (1.00)	1.00 (.192-5.197)	0.999_b	2 (4.00)	0 (.00)	–	0.062_b
HLA-A*26	13 (6.50)	29 (5.80)	1.13 (.574-2.219)	0.725_a	1 (2.00)	12 (8.00)	.23 (.030-1.852)	0.192_b
HLA-A*27	1 (.50)	0 (.00)	–	0.286_b	0 (.00)	1 (.70)	–	0.999_b
HLA-A*28	1 (.50)	1 (.20)	2.51 (.156-40.285)	0.490_a	0 (.00)	1 (.70)	–	0.999_b
HLA-A*29	3 (1.50)	6 (1.20)	1.25 (.311-5.063)	0.720_b	0 (.00)	3 (2.00)	–	0.575_b
HLA-A*30	6 (3.00)	18 (3.60)	.83 (.324-2.118)	0.693_a	2 (4.00)	4 (2.70)	1.52 (.270-8.565)	0.641_b
HLA-A*31	3 (1.50)	8 (1.60)	.94 (.246-3.567)	0.999_b	1 (2.00)	2 (1.30)	1.51 (.134-17.019)	0.999_b
HLA-A*32	12 (6.00)	26 (5.20)	1.16 (.575-2.354)	0.673_a	6 (12.00)	6 (4.00)	3.27 (1.005-10.660)	0.077_b
HLA-A*33	6 (3.00)	11 (2.20)	1.38 (.501-3.769)	0.588_a	1 (2.00)	5 (3.30)	.59 (.067-5.190)	0.999_b
HLA-A*35	1 (.50)	0 (.00)	–	0.286_b	0 (.00)	1 (.70)	–	0.999_b
HLA-A*66	0 (.00)	2 (.40)	–	0.999_a	0 (.00)	0 (.00)	–	–
HLA-A*68	7 (3.50)	26 (5.20)	.66 (.282-1.549)	0.338_a	2 (4.00)	5 (3.30)	1.21 (.227-6.432)	0.999_b
HLA-A*69	0 (.00)	2 (.40)	–	0.999_b	0 (.00)	0 (.00)	–	–
HLA-A*74	0 (.00)	1 (.20)	–	0.999_b	0 (.00)	0 (.00)	–	–

(Continues)

TABLE 2 (Continued)

B								
	COVID19 DISEASE STATUS	HOSPITALIZATION STATUS FOR COVID19						
	COVID19 + (2n = 200)	Control (2n = 500)	Statistical analysis		Hosp (+) (2n = 50)	Hosp (-) (2n = 150)	Statistical analysis	
	n - AF(%)	n - AF (%)	OR - (95%CI)	*p*	n - AF(%)	n - AF (%)	OR - (95%CI)	*p*
HLA-B*07	10 (5.00)	18 (3.60)	1.41 (.639-3.109)	0.393_a	2 (4.00)	8 (5.30)	.74 (.152-3.604)	0.999_b
HLA-B*08	5 (2.50)	17 (3.40)	.73 (.265-2.002)	0.538_a	0 (.00)	5 (3.33)	–	0.334_b
HLA-B*12	2 (1.00)	0 (.00)	–	0.081_b	0 (.00)	2 (1.30)	–	0.999_b
HLA-B*13	**16 (8.00)**	**16 (3.20)**	**2.63 (1.289-5.369)**	**0.006_a**	4 (8.00)	12 (8.00)	1.00 (.307-3.254)	0.999_b
HLA-B*14	5 (2.50)	12 (2.40)	1.04 (.363-2.999)	0.999_b	1 (2.00)	4 (2.70)	.74 (.081-6.825)	0.999_b
HLA-B*15	6 (3.00)	21 (4.20)	.71 (.280-1.775)	0.456_a	1 (2.00)	5 (3.33)	.59 (.067-5.190)	0.999_b
HLA-B*18	8 (4.00)	36 (7.20)	.54 (.245-1.177)	0.115_a	1 (2.00)	7 (4.70)	.42 (.050-3.474)	0.682_b
HLA-B*21	2 (1.00)	0 (.00)	–	0.081_b	0 (.00)	2 (1.30)	–	0.999_b
HLA-B22$_s$	**3 (1.50)**	**0 (.00)**	**–**	**0.023_b**	0 (.00)	3 (2.00)	–	0.575_b
HLA-B*27	8 (4.00)	14 (2.80)	1.45 (.597-3.503)	0.411_a	2 (4.00)	6 (4.00)	1.00 (0195-5.121)	0.999_b
HLA-B*35	30 (15.00)	105 (21.00)	.66 (.426-1.035)	0.069_a	6 (12.00)	24 (16.00)	.72 (.275-1.866)	0.493_a
HLA-B*37	2 (1.00)	3 (.60)	1.67 (.278-10.091)	0.627_b	0 (.00)	2 (1.30)	–	0.999_b
HLA-B*38	9 (4.50)	21 (4.20)	1.08 (.484-2.389)	0.859_a	3 (6.00)	6 (4.00)	1.53 (.369-6.366)	0.693_b
HLA-B*39	2 (1.00)	6 (1.20)	.83 (.166-4.155)	0.999_b	1 (2.00)	1 (0.70)	3.04 (.187-49.534)	0.438_b
HLA-B*40	8 (4.00)	23 (4.60)	.86 (.380-1.965)	0.727_a	4 (8.00)	4 (2.70)	3.17 (.763-13.196)	0.109_b
HLA-B*41	3 (1.50)	13 (2.60)	.57 (.161-2.024)	0.576_b	1 (2.00)	2 (1.30)	1.51 (.134-17.019)	0.999_b
HLA-B*44	10 (5.00)	41 (8.20)	.59 (.289-1.200)	0.141_a	4 (8.00)	6 (4.00)	2.09 (.564-7.719)	0.272_b
HLA-B*45	0 (.00)	2 (.40)	–	0.999_b	0 (.00)	0 (.00)	–	–
HLA-B*46	0 (.00)	2 (.40)	–	0.999_b	0 (.00)	0 (.00)	–	–
HLA-B*47	1 (.50)	1 (.20)	2.51 (.156-40.285)	0.490_b	0 (.00)	1 (.70)	–	0.999_b
HLA-B*48	1 (0.50)	4 (.80)	.62 (.069-5.609)	0.999_b	1 (2.00)	0 (.00)	–	0.250_b
HLA-B*49	6 (3.00)	20 (4.00)	.74 (.294-1.876)	0.527_a	2 (4.00)	4 (2.70)	1.52 (.270-8.565)	0.641_b
HLA-B*50	7 (3.50)	13 (2.60)	1.36 (.534-3.457)	0.518_a	3 (6.00)	4 (2.70)	2.33 (.503-10.787)	0.370_b
HLA-B*51	29 (14.50)	66 (13.20)	1.11 (.696-1.786)	0.650_a	10 (20.00)	19 (12.70)	1.72 (.741-4.007)	0.202_a
HLA-B*52	6 (3.00)	18 (3.60)	.83 (.324-2.118)	0.693_a	1 (2.00)	5 (3.30)	.59 (.067-5.190)	0.999_b
HLA-B*53	1 (.50)	3 (.60)	.83 (.086-8.051)	0.999_b	0 (.00)	1 (.70)	–	0.999_b
HLA-B*54	0 (.00)	1 (.20)	–	0.999_b	0 (.00)	0 (.00)	–	–
HLA-B*55	**8 (4.00)**	**7 (1.40)**	**2.94 (1.050-8.203)**	**0.042_b**	0 (.00)	8 (5.30)	–	0.205_b
HLA-B*56	1 (.50)	4 (.80)	.62 (.069-5.609)	0.999_b	1 (2.00)	0 (.00)	–	0.250_b
HLA-B*57	7 (3.50)	7 (1.40)	2.55 (.884-7.379)	0.130_b	2 (4.00)	5 (3.30)	1.21 (.227-6.432)	0.999_b
HLA-B*58	4 (2.00)	5 (1.00)	2.02 (.537-7.602)	0.285_b	0 (.00)	4 (2.70)	–	0.574_b
HLA-B*73	0 (.00)	1 (.20)	–	0.999_b	0 (.00)	0 (.00)	–	–

(Continues)

TABLE 2 (Continued)

C								
	COVID19 DISEASE STATUS	HOSPITALIZATION STATUS FOR COVID19						
	COVID19 + (2n = 200)	Control (2n = 500)	Statistical analysis		Hosp (+) (2n = 50)	Hosp (-) (2n = 150)	Statistical analysis	
	n - AF(%)	n - AF (%)	OR - (95%CI)	*p*	n - AF(%)	n - AF (%)	OR - (95%CI)	*p*
HLA-DRB1*01	17 (8.50)	30 (6.00)	1.46 (.784-2.703)	0.233_a	5 (10.00)	12 (8.00)	1.28 (.427-3.824)	0.770_b
HLA-DRB1*02	1 (.50)	0 (.00)	–	0.286_b	0 (.00)	1 (.70)	–	0.999_b
HLA-DRB1*03	19 (9.50)	45 (9.00)	1.06 (.604-1.864)	0.836_a	2 (4.00)	17 (11.30)	.33 (.073-1.464)	0.167_b
HLA-DRB1*04	38 (19.00)	92 (18.40)	1.04 (.684-1.582)	0.854_a	10 (20.00)	28 (18.70)	1.09 (.487-2.438)	0.835_a
HLA-DRB1*07	18 (9.00)	39 (7.80)	1.17 (.652-2.097)	0.600_a	6 (12.00)	12 (8.00)	1.57 (.556-4.424)	0.399_b
HLA-DRB1*08	2 (1.00)	10 (2.00)	.49 (.107-2.279)	0.524_b	0 (.00)	2 (1.30)	–	0.999_b
HLA-DRB1*09	1 (.50)	4 (.80)	.62 (.069-5.609)	0.999_b	1 (2.00)	0 (.00)	–	0.250_b
HLA-DRB1*10	7 (3.50)	10 (2.00)	1.78 (.667-4.736)	0.278_b	1 (2.00)	6 (4.00)	.49 (.058-4.170)	0.683_b
HLA-DRB1*11	37 (18.50)	121 (24.20)	.71 (.471-1.073)	0.103_a	7 (14.00)	30 (20.00)	.65 (.266-1.591)	0.344_a
HLA-DRB1*12	4 (2.00)	11 (2.20)	.91 (.285-2.883)	0.999_b	1 (2.00)	3 (2.00)	1.00 (.102-9.837)	0.999_b
HLA-DRB1*13	12 (6.00)	48 (9.60)	.60 (.312-1.157)	0.124_a	3 (6.00)	9 (6.00)	1.00 (.260-3.849)	0.999_b
HLA-DRB1*14	10 (5.00)	31 (6.20)	.80 (.383-1.656)	0.541_a	1 (2.00)	9 (6.00)	.32 (.039-2.589)	0.456_b
HLA-DRB1*15	24 (12.00)	41 (8.20)	1.53 (.896-2.601)	0.118_a	8 (16.00)	16 (10.70)	1.59 (.638-3.990)	0.315_a
HLA-DRB1*16	10 (5.00)	18 (3.60)	1.41 (.639-3.109)	0.393_a	5 (10.00)	5 (3.20)	3.22 (.892-11.635)	0.125_b

AF, allele frequency; OR, odds ratio; 95%CI, confidence interval;2n, each individual was represented by two codominant allelic data; a. Pearson Chi-Square Test; b. Fisher's Exact Test.

TABLE 3 Comparison of HLA match rates between the COVID-19 patient group and the control group in kidney transplant recipients

		COVID19 DISEASE STATUS					HOSPITALIZATION STATUS FOR COVID19(+)				
		COVID19+ (n = 100)		CONTROL (n = 250)		Statistical analysis	HOSPITALIZATION (+) (n = 25)		HOSPITALIZATION (-) (n = 75)		Statistical analysis
		n	%	n	%	*p*	n	%	n	%	*p*
HLA-A Match	n/a (Mismatch)	30	30 (β)	26	10.4 (α)	<0.001[a]	11	44	19	25,3	0.085[a]
	One Allele Match	55	55 (β)	178	71.2 (α)		13	52	42	56	
	Two Allele Match	15	15 (α)	46	18.4 (α)		1	4	14	18,7	
HLA-B Match	n/a (Mismatch)	39	39 (β)	26	10.4 (α)	<0.001[a]	10	40	29	38,7	0.684[a]
	One Allele Match	48	48 (β)	179	71.6 (α)		13	52	35	46,7	
	Two Allele Match	13	13 (α)	45	18.0 (α)		2	8	11	14,7	
HLA-DRB1 Match	n/a (Mismatch)	24	24 (β)	13	5.2 (α)	<0.001[a]	5	20	19	25,3	0.342[a]
	One Allele Match	56	56 (β)	185	74.0 (α)		17	68	39	52	
	Two Allele Match	20	20 (α)	52	20.8 (α)		3	12	17	22,7	
Total Match	n/a (Mismatch)	9	9 (β)	0	0 (α)	<0.001[a]	2	8	7	9,3	0.889[b]
	1/6 Match	18	18 (β)	3	1.2 (α)		6	24	12	16	
	2/6 Match	22	22 (α)	49	19.6 (α)		6	24	16	21,3	
	3/6 Match	33	33 (β)	127	50.8 (α)		9	36	24	32	
	4/6 Match	8	8 (α)	36	14.4 (α)		1	4	7	9,3	
	5/6 Match	1	1 (α)	8	3.2 (α)		0	0	1	1,3	
	6/6 Match	9	9 (α)	27	10.8 (α)		1	4	8	10,7	

a. Pearson Chi-Square Test; b. Fisher's Exact Test.

TABLE 4 Adjusted logistic regression model for COVID-19 infection

	COVID19 DISEASE STATUS				
		COVID19(+)	Control	Statistical Analysis	
		n	n	OR (Univariable)	OR (Multivariable)
AGE		–	–	–	1.01 (.99-1.02, $p = 0.238$)
BLOOD TACROLIMUS LEVEL		–	–	–	1.05 (1.00-1.11, $p = 0.067$)
HLA-B*13		16	16	2.63 (1.29-5.37, $p = 0.006$)	2.15 (.92-4.94, $p = 0.074$)
HLA-B*18		8	36	.54 (.25-1.18, $p = 0.115$)	.45 (.18-1.01, $p = 0.068$)
HLA-B*35		30	105	.66 (.43-1.04, $p = 0.069$)	.61 (.35-1.02, $p = 0.067$)
HLA-B*44		10	41	.59 (.29-1.20, $p = 0.141$)	.61 (.26-1.35, $p = 0.248$)
HLA-B*55		8	7	2.94 (1.05-8.20, $p = 0.042$)	1.59 (.46-5.52, $p = 0.462$)
HLA-B*57		7	7	2.55 (.88-7.38, $p = 0.130$)	3.58 (1.10-11.64, $p = 0.031$)
HLA-DRB1*11		37	121	.71 (.47-1.07, $p = 0.103$)	.60 (.37-.97, $p = 0.042$)
HLA-DRB1*13		12	48	.60 (.31-1.16, $p = 0.124$)	.43 (.19-.90, $p = 0.033$)
HLA-DRB1*15		24	41	1.53 (.89-2.60, $p = 0.118$)	1.40 (.74-2.60, $p = 0.295$)
HLA-A Match	n/a (Mismatch)	30	26		
	One Allele Match	55	178	.27 (.17-.41, $p < 0.001$)	.40 (.24-.66, $p < 0.001$)
	Two Allele Match	15	46	.28 (.16-.49, $p < 0.001$)	.39 (.18-.83, $p = 0.015$)
HLA-B Match	n/a (Mismatch)	39	26		
	One Allele Match	48	179	.18 (.12-.27, $p < 0.001$)	.23 (.14-.37, $p < 0.001$)
	Two Allele Match	13	45	.19 (.11-.33, $p < 0.001$)	.31 (.14-.65, $p = 0.002$)
HLA-DRB1 Match	n/a (Mismatch)	24	13		
	One Allele Match	56	185	.16 (.10-.27, $p < 0.001$)	.20 (.11-.37, $p < 0.001$)
	Two Allele Match	20	52	.21 (.11-.38, $p < 0.001$)	.28 (.13-.59, p = 0.001)

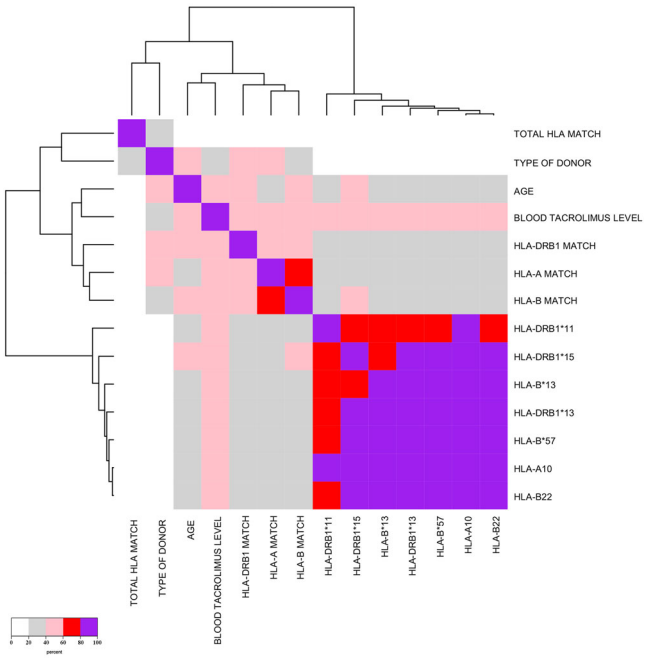

FIGURE 1 Heatmap of the variable similarity matrix of COVID-19 patients with statistically significant variables from the univariate analysis

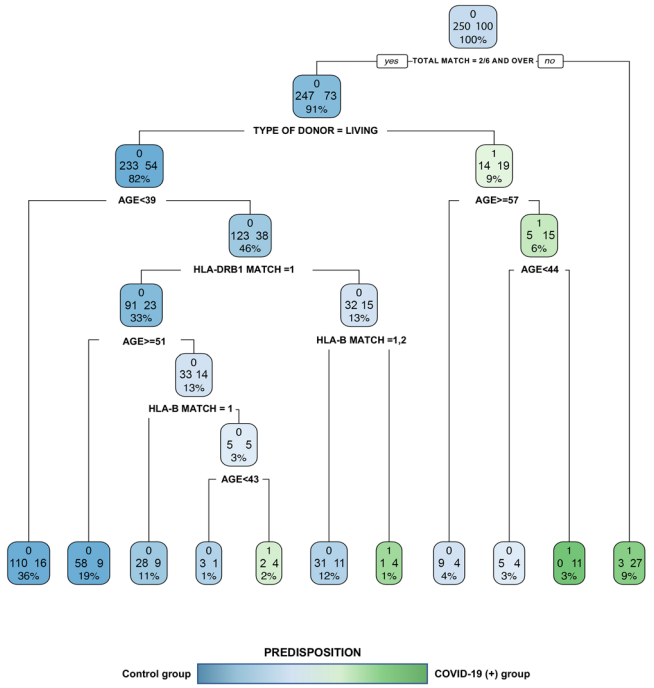

FIGURE 2 Decision tree with statistically significant variables from the univariate analysis *(Data in nodes; the number above indicates whether the majority of COVID-19(+) or COVID-19(-) is in that node (1 or 0). Middle right number shows the number of COVID-19(+) volunteers in that group. Middle left number shows the number of COVID-19(-) volunteers in that group. The number below shows the percentage of individuals in that group in the entire sample.)*

3.5 | Decision tree

In the decision tree, the incidence of COVID-19 disease is higher in four out of 11 terminal nodes compared to the control group (Figure 2). In cases where the HLA match is not 2/6 or more, the number of individuals with COVID-19(+) is 27, while the number of COVID-19(-) individuals is only three. In cases where the HLA match is 2/6 and over, all deceased donor recipients over the age of 44 appear to be infected with COVID-19. Only 16 out of 126 living recipients aged under 39 were infected with SARS-CoV-2. In terms of living donor recipients older than 51, having a match for HLA-DRB1 reduces the incidence of COVID-19 (58/9). The decision tree we display presents only the main features of the tree. As can be seen in Figure 2, features such as HLA-B*57, HLA-A10, HLA-B*11, HLA-DRB1*13 do not appear in the tree. Trees can be enlarged by changing the parameters of *rpart* function to have a more detailed tree with many more nodes and leafs, but this would make the interpretation cumbersome. Still the *rpart* function may not show all the variables in the data, depending on the significance of each split.

4 | DISCUSSION

Our study aimed to investigate the relationship between HLA genotyping and COVID-19 infection in kidney transplant recipients. In addition to HLA, the relationship between COVID-19 and parameters such as age, gender, etiology of CKD, donor type, rejection, and drugs were also investigated. Our study includes one of the most comprehensive kidney transplant series on COVID-19 disease in the literature.[18]

A statistically significant difference was observed in this study between the patient and control groups HLA-match rates. In terms of the HLA-A match, the one allele match rate was higher among living donor recipients than in the deceased group. In contrast, more than 50% of deceased donor recipients had 2/6 or less of the total match rate (Supplementary Information 1). In addition, cold-ischemia time and other immunological factors which were not evaluated within the scope of this study should also be investigated.

As expected, the mean age of the COVID-19(+) patient group was higher than that of the control group, consistent with the previous literature.[8,19] However, in contrast to previous studies, the donor type was statistically significant in terms of COVID-19 infection in the present study.[20] In seeking to explain this based on the data obtained, the mean ages of the living and deceased donor groups were significantly different (Supplementary Information 1).

A statistical relationship was observed between the rate of COVID-19-related hospitalization and the type of donor. In addition, blood tacrolimus levels also exhibited a statistically significant relationship with hospitalization. A high blood tacrolimus level increases the recipient's likelihood of hospitalization. Tacrolimus use has been associated with better survival in SARS-CoV-2-dependent infections in the literature and seems to have positive effects on the morbidity caused by a

cytokine storm.[21] Only two of our patients had severe COVID-19 disease, the other cases being mild to moderate. The difference between the literature and the findings of the present study could be due to the clinical stage difference. Tacrolimus may adversely affect mild and moderate COVID-19 disease due to its effects on the immune system, while exerting a positive effect in severe COVID-19 disease through its inhibition of the cytokine storm.

Univariate analysis revealed higher HLA-A10, HLA-B*13, HLA-B22, and HLA-B*55 allele frequencies in patients infected with COVID-19 compared to the control group. HLA-A10 is a group named after serological typing. HLA-A10 is divided into five subgroups according to its new genotype nomenclature HLA-A*25, HLA-A*26, HLA-A*34, HLA-A*43, and HLA-A*66.[22] The previous study already showed that the frequency of the HLA-A*25 allele, which is one of the subgroups of HLA-A10, showed a positive log-linear correlation with the COVID-19 incidence rate.[9] On the other hand, the HLA-A10 group is no longer defined since this was a method previously used in our serological typing laboratory. Due to the age of this method, the average age of the recipients using it is high, considering the time elapsed. The high average age of the recipients may have contributed to the statistical significance of this subgroup. In addition, the frequency of HLA-A10 detection has decreased because the HLA-A10 subgroup has not been detected recently. The statistical significance is likely to be high due to the lack of frequency in the control group. Also, in the literature, a study describing the SARS-CoV-2 peptide presentation by A*26:01, a subgroup of the HLA-A10 serological group.[23]

To the best of our knowledge, similarly to the HLA-A10 subgroup, the HLA-B22 subgroup is obtained by serological typing. The HLA-B22 serological subgroup includes the HLA-B*54, HLA-B*55, and HLA-B*56 genotypes. A study from China reported that the frequency of the HLA-B22 serological group was statistically significantly associated with COVID-19 infection.[24] Consistent with that research, the results of the present study also showed that HLA-B22 is associated with COVID-19. In addition, the frequency of the HLA-B*55 genotypic subgroup was found to be related to COVID-19 in the present study.

HLA compatibility is analyzed in the decision-making phase of kidney transplants. The relationship between HLA compliance and COVID-19 infection was evaluated within the scope of the project. After the analysis, the mismatch in the HLA-A groups provides statistically significant sensitivity to COVID-19 infection. This significance is also valid for HLA-B and HLA-DRB1. However, matching one allele in the HLA-A, HLA-B, or HLA-DRB1 groups has been observed to significantly reduce the incidence of COVID-19 disease. This finding is reported for the first time in the present study. The presence of a HLA-match could be considered to positively affect the immune system in terms of COVID-19 disease, with the possibility of developing anti-HLA against another HLA subgroup. Leith et al. demonstrated that the allele-specific anti-HLA antibody neutralizes the HIV-1 virus in vitro.[25] Another study showed virus neutralization of anti-HLA antibodies.[26] Anti-HLA antibodies were likely to effectively neutralize the SARS-CoV-2 virus, another cause of viral infection, as with the HIV-1 virus. In addition, *CXCL9*, *CXCL10*, and *CCL8* genes have been reported to exhibit high expression in mismatched mixed lymphocyte cultures.[27] The same gene and gene products (chemokines) are also involved in COVID-19 disease, suggesting the possibility of common molecular pathways between HLA-mismatches and SARS-CoV-2 virus infection.[28] Finally, studies have shown that multiple-dose influenza vaccine increases anti-HLA antibodies. These findings strongly suggest a potential relationship between viral infection immunity and anti-HLA antibodies.[29] The relationship between the anti-HLA antibody and the inflammation response created by the SARS-CoV-2 virus needs to be investigated in future studies.

Evaluation of HLA frequencies and COVID-19-associated hospitalization status revealed that HLA-A*11 was statistically significant. In-silico analysis reported in the previous literature showed that the HLA-A*11 alleles may be associated with hospitalization in COVID-19 infection.[30] The analyses performed in the present study showed that this retrospective in silico analysis is clinically accurate. A study from Spain found that the HLA-A*11 alleles were associated with high mortality.[11] Considering that the patients were hospitalized due to poor prognosis of COVID-19 disease (WHO scores 6–9), this seems to be consistent with our findings concerning the HLA-A*11 allele.

The present study shows, for the first time in the literature, that HLA-A*13, HLA-B*57, HLA-DRB1*11, and HLA-DRB1*13 are associated with SARS-CoV-2 infection. Previous studies have shown that these alleles are associated with viral and bacterial infection.[31–34] Additionally, the conclusions are based on a single center study and further investigation in other populations is needed to confirm these associations.

It is very important to analyze the SARS-CoV-2 peptide binding capacities of the HLA subtypes that we found associated with the SARS-CoV-2 viral infection in this study. In the literature, the binding capacity of HLA-A*26, which is the genetic subgroup of HLA-A10, to SARS-CoV-2 peptide has been investigated.[23] In addition, it has been stated in the literature that different HLA-B correlations may show different affinity for the SARS-CoV-2 peptide, which may be associated with daily deaths related to COVID-19.[35] Investigation of peptide binding capacities of other subgroups is also very important in the fight against COVID-19 infection.

In addition, in the study conducted with the in silico analysis method in the literature, the relationship between Covid and HLA in our country was determined. In that study, the subtype with the highest HLA allele frequency is HLA A*02. Although there are other studies, including Turkey, examining the HLA-COVID19 relationship,[36] as is known, our country has a heterogeneous structure. This study has the feature of having the largest cohort showing the Covid-19&HLA relationship covering kidney transplant individuals covering the Mediterranean region in Turkey.

One of the limitations of the present study is that we were unable to analyze the anti-HLA antibody since the genetic data were obtained retrospectively, and anti-HLA antibody data were not available for all patients. For the same reason, we were unable to analysis SARS-CoV-2 IgM and IgG antibody levels. Additionally, since the tissue typing results were evaluated retrospectively, this enabled us to access the serological typing results of some HLA alleles, HLA-A10 and HLA-B22. Lack of genotypic typing of statistically significant HLA alleles is a further lim-

itation of this study. Finally, this study involved only kidney transplant recipients, and it is important that similar studies be performed in other SOTs.

In our study, we can say that more statistical tests were performed than should be due to the large number of variables. This situation can lead to multiple testing (multiplicity) problem and this problem may highly increase random variation and type-I error.[37]

According to ICH E9 guideline, to avoid this problem, we specified in the protocol the precise definition of the primary variable.[38] We did not change the statistical methods following finalization of the protocol and statistical analysis were pre-planned. On the other hand, our primary outcomes consisted of only COVID-19(+) and hospitalization. In subgroup testing through a post hoc evaluation, we use Bonferroni correction to avoid type I error.

Finally, as this study is retrospective in nature, all results are associative and should be validated in future studies.

In conclusion, this study examined COVID-19 infection susceptibility in kidney transplant recipients. Demographic characteristics such as age, and some subgroups of HLA were found to exhibit statistically significant associations with COVID-19 infection. A relationship between HLA match rates and COVID-19 was also shown for the first time in this study. These data suggest the possibility of a relationship between anti-HLA antibody and SARS-CoV-2 infection, as with other viral agents.

Due to the differences in HLA prevalences among different geographic regions, there is a strong likelihood that new research may yield different results regarding SARS-CoV-2 infection in transplant patients. It is crucial that new information be elicited by conducting such studies in different geographic regions in order to improve the global healthcare of transplant patients in a proactive manner.

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CONFLICTS OF INTEREST

None.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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