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CELLULAR AND MOLECULAR BIOLOGY

HLA haplotypes and differential regional mortality caused by COVID-19 in Brazil: an ecological study based on a large bone marrow donor bank dataset

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Abstract: The coronavirus disease 2019 (COVID-19) mortality rates varied among the states of Brazil during the course of the pandemics. The human leukocyte antigen (HLA) is a critical component of the antigen presentation pathway. Individuals with different HLA genotypes may trigger different immune responses against pathogens, which could culminate in different COVID-19 responses. HLA genotypes are variable, especially in the highly admixed Brazilian population. In this ecological study, we aimed to investigate the correlation between HLA haplotypes and the different regional distribution of COVID-19 mortality in Brazil. HLA data was obtained from 4,148,713 individuals registered in The Brazilian Voluntary Bone Marrow Donors Registry. COVID-19 data was retrieved from epidemiological bulletins issued by State Health Secretariats via Brazil's Ministry of Health from February/2020 to July/2022. We found a positive significant correlation between the HLA-A*01~B*08~DRB1*03 haplotype and COVID-19 mortality rates when we analyzed data from 26 states and the Federal District. This result indicates that the HLA-A*01~B*08~DRB1*03 haplotype may represent an additional risk factor for dying due to COVID-19. This haplotype should be further studied in other populations for a better understanding of the variation in COVID-19 outcomes across the world.

Key words: COVID-19 variability, COVID-19 mortality, Genetic susceptibility, SARS-CoV-2.

INTRODUCTION

Brazil surpassed 670,000 deaths due to coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2 (WHO 2022). The mortality rates varied considerably among the Brazilian regions during the course of pandemics (CORONAVIRUS BRASIL 2020). This variability could be partially explained by the patient clinical profile and unequal access to the health system in different regions (Li et al. 2020, Zhou et al. 2020). In addition to the social and environmental conditions, genetic factors may also influence susceptibility and immune response to SARS-CoV-2 infection. Many studies are investigating genetic factors that could influence on COVID-19 susceptibility and severity worldwide. Several of them have focused on the variability of the genetic complex of the classic human leukocyte antigen (HLA), a critical component of the antigen presentation pathway. Individuals with different HLA genotypes may trigger different immune responses against SARS-CoV-2 (Dendrou et al. 2018, Saghazadeh 2020). The HLA variability observed worldwide

is accentuated in Brazil, since each Brazilian region has a particular admixture history and different genetic backgrounds (Gonzalez-Galarza et al. 2020).

In a recent review, Migliorini et al. (2021) showed that a few HLA alleles or haplotypes may have a protective effect or an increased susceptibility to SARS-CoV-2 infection. However, these studies had a limited number of patients and the associated alleles varied depending on the population under investigation. Additionally, *in silico* studies have analyzed the binding affinity between the SARS-CoV-2 peptides and different HLA class I genotypes (Barquera et al. 2020, Kiyotani et al. 2020, Nguyen et al. 2020, Tomita et al. 2020). In Italy, two studies compared HLA allele prevalence retrieved through the Italian Bone-Marrow Donor Registry with the incidence of SARS-CoV-2 infections in the different geographical regions of the country. This strategy increases the number of analyzed individuals and could be very informative, even though the HLA genotypes are not obtained directly from COVID-19 patients (Correale et al. 2020, Pisanti et al. 2020).

The diverse results obtained from HLA and COVID-19 studies highlight the need for additional analyses in different populations. In the present study, we investigated a large sample of HLA haplotypes from all Brazilian states retrieved from The Brazilian Voluntary Bone Marrow Donors Registry (REDOME, *Registro de Doadores de Medula Óssea*), the third largest bone marrow donors bank in the world, and compared to COVID-19 data in the correspondent region.

MATERIALS AND METHODS

HLA data was retrieved from a dataset composed by 4,148,713 individuals, who registered at REDOME until September, 2017. This registry includes donors' city of residence and HLA-A, -B, and -DRB1 genotypes at allelic resolution level. Donors came from recruitment centers distributed throughout the country and their DNA was genotyped in Health Ministry accredited Brazilian laboratories. The HLA dataset was subdivided according to state and city of residence (Brazilian territory is divided into 26 states and one Federal District). The study was approved by the Committee of Ethics in Research of Hospital de Clínicas de Porto Alegre, Brazil.

COVID-19 number of cases and deaths were obtained from the epidemiological bulletins issued by State Health Secretariats via the Ministry of Health of Brazil (Fiocruz 2022) from February/2020 to July/2022. The COVID-19 incidence and mortality coefficients were calculated for each state. Estimations of HLA allele and haplotype frequencies, and Hardy-Weinberg equilibrium (HWE) test were performed using the GENE [RATE] tools (Boquett et al. 2017, Buhler et al. 2012, Nunes 2015). Maps of COVID-19 mortality rates were generated in ArcGis v10.3. Spearman's correlation test between the five most frequent haplotypes in Brazil (Torres et al. 2017) and COVID-19 mortality rates in the 26 states and the Federal District was performed using IBM SPSS software, Version 20.0 (IBM Corp., Armonk, NY). P-values below 0.05 were considered statistically significant. Bonferroni's correction for multiple tests was applied for haplotype correlations ($\alpha_{Bonf} = 0.01$).

RESULTS

The geographical distribution of the COVID-19 epidemic in Brazil in our analysis was as follows: the states with the highest incidence of COVID-19 cases registered until July 6th, 2022 were Espírito Santo, the Federal District and Roraima, respectively; while the states with the lowest incidence were Maranhão, Pará and

Alagoas (Table I). Regarding COVID-19 mortality, Rio de Janeiro, Mato Grosso and Rondônia had the highest rates, respectively, while the lowest mortality rates were observed in Maranhão, Bahia and Alagoas, respectively (Table I, Figure 1).

HLA allele frequencies did not deviate from HWE expectations and were used to estimate the haplotypes. The five most frequent HLA-A~B~DRB1 haplotypes found in the analyzed population are presented in Table II. The most frequent haplotype was HLA-A*01~B*08~DRB1*03, with a frequency ranging from 1.2% in Pará, Maranhão and Amapá, states located in the North (Pará, Amapá) and Northeast (Maranhão) of the country, to 3.1% in Rio Grande do Sul and Santa Catarina, both states located in the South. Rio Grande do Sul

Table I. Regional data relative to the impact of COVID-19 on the Brazilian population until July 6th, 2022.

State	Region	Cases/100,000 inhabitants	Deaths/100,000 inhabitants
Distrito Federal	CW	26613.95	385.81
Goiás	CW	21400.72	378.77
Mato Grosso	CW	21781.43	418.01
Mato Grosso do Sul	CW	19691.29	378.91
Alagoas	NE	9196.61	207.87
Bahia	NE	10657.9	201.26
Ceará	NE	13848.91	296.34
Maranhão	NE	6241.42	153.19
Paraíba	NE	15433.28	254.23
Pernambuco	NE	10260.47	227.64
Piauí	NE	11404.34	237.02
Rio Grande do Norte	NE	15014.35	234.23
Sergipe	NE	14304.63	274.3
Acre	NO	14277.17	224.16
Amapá	NO	18836.4	248.44
Amazonas	NO	13957.96	336.98
Pará	NO	9027.44	212.56
Rondônia	NO	23436.59	403.63
Roraima	NO	25859.62	341.11
Tocantins	NO	20400.84	262.1
Espírito Santo	SE	28007.49	357.43
Minas Gerais	SE	17191.25	292.28
Rio de Janeiro	SE	13641.74	427.45
São Paulo	SE	12430.68	369.86
Paraná	SO	22898.3	380.86
Rio Grande do Sul	SO	22421.23	351.05
Santa Catarina	SO	24827.46	304.21

CW: Central-West; NE: Northeast; NO: North; SE: Southeast; SO: South.

and Santa Catarina had a high outlier frequency for haplotypes #1 (HLA-A*01~B*08~DRB1*03) and #3 (HLA-A*03~B*07~DRB1*15) (Figure 2).

Spearman correlation coefficient was calculated to test if the regional COVID-19 mortality correlated with any of the five most frequent haplotypes in Brazilian population. We found a strong and significant correlation (*rho* = 0.687, P < 0.001) between the haplotype HLA-A*01~B*08~DRB1*03 frequency and COVID-19 mortality rates for the 26 states and the Federal District (Table III). No significant correlation was found in the remaining haplotypes analyzed.

DISCUSSION

Many studies have employed different approaches to investigate the role of the HLA system in COVID-19, such as *in silico* (Barquera et al. 2020, Kiyotani et al. 2020, Nguyen et al. 2020, Tomita et al. 2020), case-control (Kousathanas et al. 2022, Langton et al. 2021, Wang et al. 2020) and ecological (Correale et al. 2020, Pisanti et al. 2020). In this ecological study, we found a significant positive correlation between the HLA- A*01~B*08~DRB1*03 haplotype and COVID-19 mortality rates when analyzing data from the 26 states and the Federal District (Table III). Pisanti et al. (2020) also found a significant correlation between the two most frequent haplotypes in the Italian population with both COVID-19 incidence and mortality using a similar ecological approach. In their study, the haplotype HLA-A*01:01g~B*08:01g~C*07:01g~DRB1*03:01g showed a positive correlation with both COVID-19 incidence and mortality.

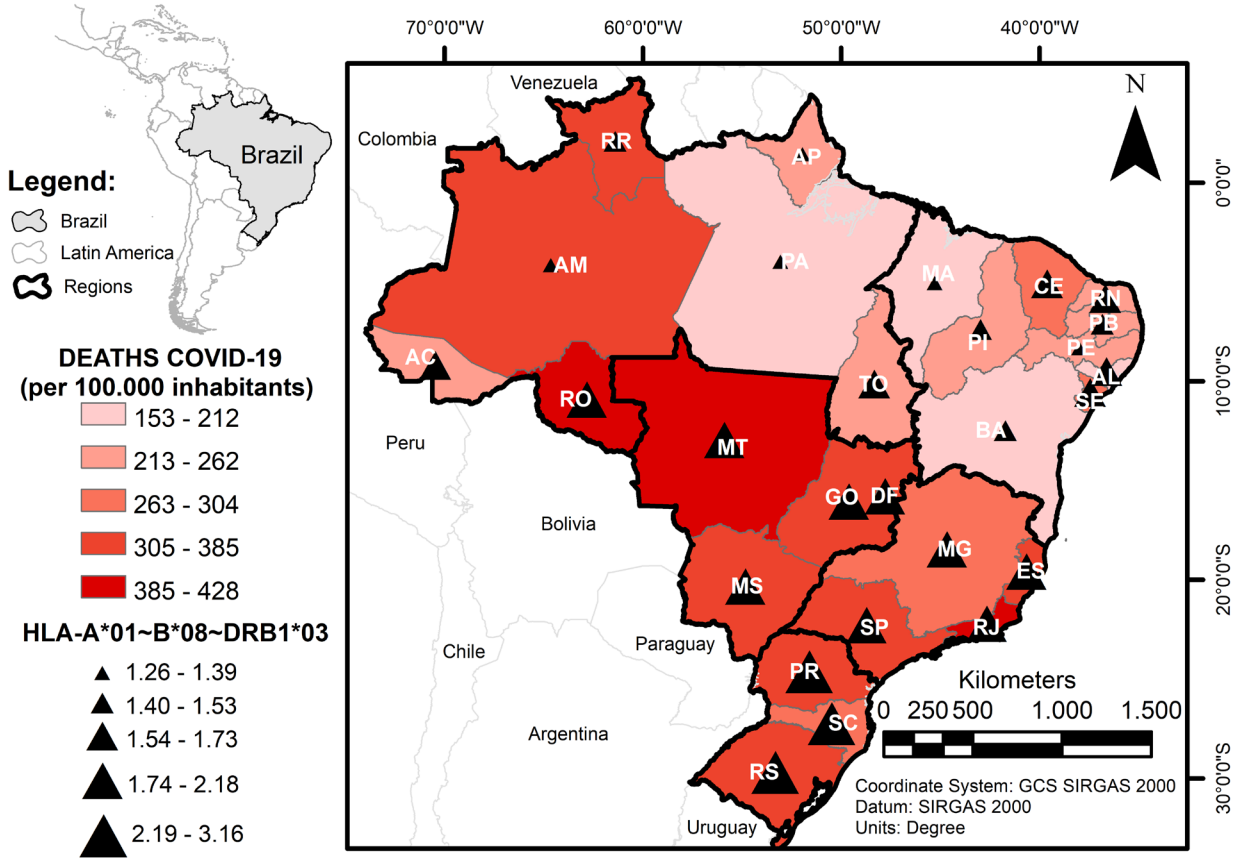

Figure 1. COVID-19 deaths per 100,000 inhabitants in different Brazilian states until July 6th, 2022, and HLA-A*01~B*08~DRB1*03 haplotype frequency in the 26 Brazilian states and Federal District.

In Brazil, we observed that the four most common HLA haplotypes had higher frequencies in the South region and lower frequencies in the North (Table II). These regional differences reflect the different contributions of Native American, European, and African populations across the country after a long history of colonization and immigration (IBGE 2020). This is the first study in the country evaluating HLA haplotype frequencies and COVID-19 mortality rates. A previous descriptive study in Brazil focused on HLA alleles retrieved from Brazilian exome databases and compared the frequency of these HLA alleles with populations that

Table II. Frequencies (%) of the five most common haplotypes observed in the Brazilian population.

State	Region	#1 HLA-A*01~B*08 ~DRB1*03	#2 HLA-A*29~B*44 ~DRB1*07	#3 HLA-A*03~B*07 ~DRB1*15	#4 HLA-A*02~B*44 ~DRB1*04	#5 HLA-A*33~B*14 ~DRB1*01
Distrito Federal	CW	1,97	1,53	0,97	0,83	0,92
Goiás	CW	2,16	1,69	0,81	0,90	0,76
Mato Grosso	CW	2,13	1,38	0,95	0,87	0,80
Mato Grosso do Sul	CW	2,03	1,39	0,97	0,86	0,73
Alagoas	NE	1,71	1,29	0,98	0,95	0,93
Bahia	NE	1,50	1,19	0,77	0,87	0,82
Ceará	NE	1,73	1,55	0,86	0,75	0,85
Maranhão	NE	1,27	1,25	0,75	0,79	0,74
Paraíba	NE	1,53	1,49	1,21	1,01	0,80
Pernambuco	NE	1,39	1,28	0,96	0,82	0,91
Piauí	NE	1,52	1,38	0,90	0,77	0,85
Rio Grande do Norte	NE	1,71	1,42	0,90	1,17	0,62
Sergipe	NE	1,65	1,22	0,61	0,87	0,59
Acre	NO	1,67	1,41	0,81	0,76	0,72
Amazonas	NO	1,30	1,26	0,61	0,65	0,79
Amapá	NO	1,29	1,31	0,63	0,77	0,46
Pará	NO	1,26	1,29	0,68	0,76	0,60
Rondônia	NO	2,05	1,39	1,01	0,84	0,85
Roraima	NO	1,52	1,25	0,77	0,67	0,80
Tocantins	NO	1,61	1,58	0,87	0,82	0,75
Espírito Santo	SE	2,12	1,39	1,02	0,79	0,80
Minas Gerais	SE	2,11	1,65	0,89	0,96	0,85
Rio de Janeiro	SE	1,91	1,54	1,03	0,93	0,83
São Paulo	SE	2,18	1,46	0,93	0,74	0,83
Paraná	SO	2,69	1,39	1,27	0,75	0,79
Rio Grande do Sul	SO	3,16	1,64	1,48	0,98	0,61
Santa Catarina	SO	3,13	1,72	1,66	1,18	0,70

CW: Central-West; NE: Northeast; NO: North; SE: Southeast; SO: South.

occupy the top 10 positions for most cases of COVID-19 and the five populations less affected by the disease (Secolin et al. 2021). They found that the HLA alleles *HLA-DQB1**06:02 and *HLA-DRB1**15:01 were frequent in Brazil and in less affected populations.

Together with genetic and immune system variation, environmental and social disparities among Brazilian regions may contribute to the differential burden of COVID-19, disproportionately affecting individuals carrying genetic factors of susceptibility and/or the most vulnerable people regarding social assistance. As the number of reported cases depends on the number and type of diagnostic tests performed by each city and state, and with limited testing it is unlikely that asymptomatic subjects would have been diagnosed for SARS-CoV-2 infection, these numbers should be taken with caution. In this sense, the number of COVID-19 related deaths used in this study could be more reliable.

Distinguishing among genetic, social and environmental variability, though challenging, is essential for building an efficient way to prevent, control and understand the disease (Zhao et al. 2019). In this sense, our study is an important initial step in the understanding of COVID-19 dispersion and behavior in Brazil. Overall, our analysis provided support for the association of HLA haplotype and a severe outcome from COVID-19. An important limitation of our study is that we used HLA data from bone

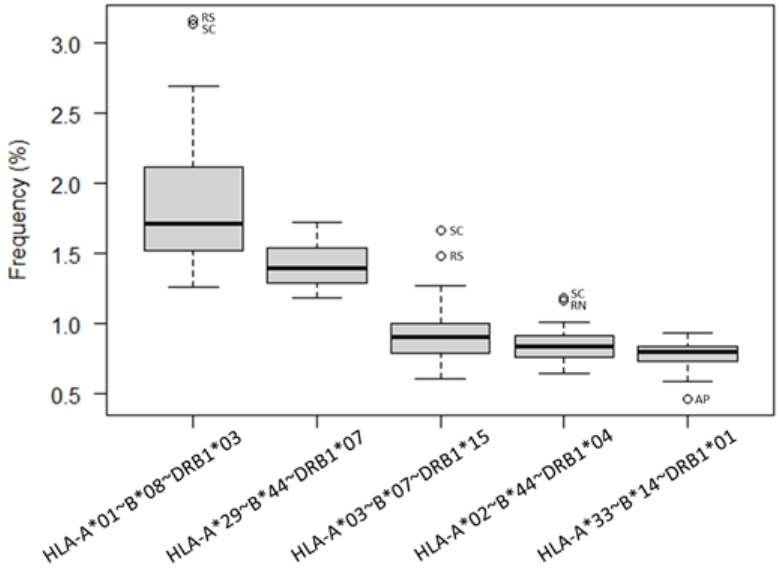

Figure 2. Box-and-whisker plots with the five most frequent HLA-A~B~DRB1 haplotypes in Brazil. AP: Amapá; RN: Rio Grande do Norte; RS: Rio Grande do Sul; SC Santa Catarina.

Table III. Bivariate correlation analysis among regional haplotypes estimated frequency in the population and COVID-19 and mortality (N° deaths/100,000 inhabitants) in Brazilian states until July 6th, 2022.

Haplotypes	*rho*	P
A*01~B*08~DRB1*03	0,687	0,0001*
A*29~B*44~DRB1*07	0,422	0,0284
A*03~B*07~DRB1*15	0,463	0,0149
A*02~B*44~DRB1*04	-0,002	0,9940
A*33~B*14~DRB1*01	0,157	0,4345

***rho* = Spearman Correlation Coefficient. *Remained significant after Bonferroni's correction.**

marrow donors instead of directly genotyping individuals affected by COVID-19. However, this approach has some advantages. Bone marrow donor registries usually include very large sample sizes and a wide geographic coverage, as illustrated by the REDOME registry. Ecological studies based on databases such as this one are a cost-effective and useful alternative for raising hypotheses, which could be tested in further studies with patients. Similar studies have been performed with REDOME and other diseases databases in the Brazilian population (Boquett et al. 2018). In addition, because several countries maintain such large banks, statistically significant associations could reveal regions or populations in higher genetic risk for COVID-19, thus representing an additional tool for health policymakers in the fight against COVID-19.

In conclusion, in this ecological approach, we found a significant positive correlation between the HLA- A*01~B*08~DRB1*03 haplotype and COVID-19 mortality rates when analyzing data from the Brazilian 26 states and the Federal District. The probable association of the HLA haplotype with a severe outcome from COVID-19 in Brazil could be useful to identify more vulnerable populations and guide public policies and vaccination strategies in case of novel disease waves.

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Author contributions

JAB and CR conceived and designed the study; JAB and LS conducted the experiments; JAB, FSLV, NJRF, MB, MZO, TFA and LCMSP analyzed the data, participated in formal analysis and critical review; JAB, JABC, LSF, PAP and CR wrote the manuscript. All authors read and approved the final version.