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Frequency of classes I and II HLA alleles in deceased donors of solid organs in Espírito Santo, Brazil

Frequência alélica HLA de classes I e II em doadores falecidos de órgãos sólidos no Espírito Santo, Brasil

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ABSTRACT

Introduction: The Brazilian population has a great racial mix, originating mainly from Europeans, Africans and Indians. Human leukocyte antigens (HLA) specificities vary among different population and ethnic groups, since they demonstrate greater similarity in populations of common origin. **Objective:** To determine the genetic structure of HLA classes I and II alleles in deceased donors of solid organs in the state of Espírito Santo, Brazil, from 2011 to 2015. **Methods:** The present study covered 208 brain-dead donors of solid organs, from 2011 to 2015, in the state of Espírito Santo, Brazil. HLA typing was performed by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSP) using kits for generic classes I and II SSP DNA typing test, A, B and DR *loci*. **Results:** The profile of deceased donors found in Espírito Santo, Brazil, in the period from 2011 to 2015, was brown men, aged over 40 years. The most frequent HLA alleles were A2 (24.28%), B44 (10.34%) and DR15 (14.18%); and the least frequent, A43 (0.24%), B46, B47, B70, B75 and B78 (0.24%) and DR9 (1.68%), DR12 and DR18 (2.16%). **Conclusion:** The present study contributed with significant information about the frequency of HLA antigens in deceased donors of solid organs in the Brazilian population that will contribute to optimize the transplantation process in the country.

Key words: tissue donors; alleles; HLA antigens.

INTRODUCTION

The human leukocyte antigen (HLA) system is encoded by the major histocompatibility complex (MHC), and comprises a group of genes located on a small segment of chromosome 6⁽¹⁾, being divided into three subregions: class I (A, B, and C), class II (DR, DQ, and DP), and class III⁽²⁾. Class I and II genes define tissue compatibility, influencing the success of organ and tissue transplantation⁽³⁾. Organ transplantation is a complex procedure aimed at improving the quality of life of individuals that present a chronic irreversible disease⁽⁴⁾.

HLA antigens are cell surface glycoproteins involved in the regulation of immune response. Those molecules are highly polymorphic, differing between individuals and population groups⁽²⁾. They are engaged in the allorecognition of antigens in organ transplantations that trigger lymphocyte activation and the graft rejection process^(3, 5). The function of MHC molecules is to

exhibit peptide fragments of protein for recognition by antigen-specific T lymphocytes⁽⁶⁾.

MHC genes are codominantly expressed in each individual, increasing the number of HLA molecules available to present peptides to T cells. Thus, in the global population, there are several alleles of each MHC gene, and each individual inherits a group of these alleles that is different from the alleles of most other individuals⁽⁷⁾. Even with the advances in medicine, HLA sensitization and ABO blood group incompatibility are significant barriers to organ donation, and can cause hyperacute rejection and graft loss⁽⁴⁾.

The existing legislation requires conduction of a genetic test for HLA typing prior to transplantation. It is one of the most important tests to evaluate compatibility between donor and recipient, because the greater the compatibility, the greater the chances are of transplant success, patient and graft survival⁽⁸⁾. In clinical practice, compatibility among class II HLA-DR antigens is highly important, followed by compatibility among HLA-B antigens,

and, lastly, class I HLA-A antigens. *C locus* antigens are of little importance regarding rejection in solid organ transplantation, for they seem little antigenic⁽⁹⁾.

After evaluating genetic compatibility, another test to be carried out is the cross-match, employed to determine if the recipient is not sensitized against the donor's histocompatibility antigens. This test must result negative for transplantation to be possible. A negative cross-match enables transplantation⁽¹⁰⁾.

The search for donors among relatives must be encouraged, for the probability of finding a compatible related donor is higher than that of finding a deceased donor, besides the chance of a better match among family members, what prolongs graft and recipient survival^(11,12).

There has been a considerable decline in the number of living kidney donors in the United States. Insufficient information for organ donors and recipients, different procedures among the different ethnicities, economic difficulties, and lack of public policies and health professionals are some factors that limit living organ donations. A recent consensus on live donor kidney transplantation brings especially educational recommendations to minimize difficulties⁽¹³⁾.

In Brazil, patients need to be registered at the Center for Organ Sharing at their region to be entitled for a deceased organ donation. It is a single waiting list system, which guarantees equity of access, but, given the necessity of compatibility between donor and recipient, not always the patient who ranks first on the list will be the next one to receive a graft⁽¹⁴⁾.

The Brazilian population has a great racial mix and was mainly originated by Europeans, Africans, and Indigenous people⁽¹⁵⁾. HLA specificities vary between the different population and ethnic groups, as they demonstrate higher similarity between populations with common origin⁽¹⁶⁾. So, ethnic diversity in the country hampers the identification of non-related genetically similar individuals according to HLA.

The state of Espírito Santo comprises a territory of 46,086.907 km², with 78 municipalities and an estimated population of 3,973,697 inhabitants in the year 2016 [Instituto Brasileiro de Geografia e Estatística (IBGE)]⁽¹⁷⁾.

For the conduction of solid organ transplantations, several criteria are considered, especially compatibility between parts, so that treatment is safe⁽⁵⁾. Brazil lacks recent studies on the genetic profile of individuals, what delays the beginning of transplantations in the country. Thus, we want to genetically characterize the deceased organ donors in Espírito Santo to learn their profiles and improve the search for compatible donors.

OBJECTIVE

Determine the genetic structure of HLA alleles classes I and II in solid organ deceased donors in the state of Espírito Santo, Brazil, between 2011 and 2015.

METHODS

The current study was conducted in brain-dead donors of solid organs, from 2011 to 2015, in the state of Espírito Santo, Brazil. Analyses were carried out at Laboratório de Biologia Molecular e Imunogenética Ltda. in the city of Vitória, Espírito Santo, Southeast region of Brazil.

Sampling was performed by the convenience method; it was composed of 208 patients: 76 (36.5%) women and 132 (63.5%) men. Demographic data were collected by analysis of patients' records in the laboratory databank, through a computerized internal system. Sex, age, and ethnic-racial classification were analyzed so as to design donors' profiles, additionally to the information on genetic diversity. Donors without registry number and from periods before or after the study were excluded, as well as those from other states of Brazil.

Sample collection and extraction of deoxyribonucleic acid (DNA)

Samples of peripheral blood (8 ml) were collected in Vacutainer tubes, containing ACD-A anticoagulant. DNA was extracted from blood samples using the Biopur extraction kit Mini Spin® column [Biometrix Diagnóstica, Curitiba (PR), Brazil], according to the manufacturer's instructions.

PCR-SSP typing of HLA class I and II alleles

HLA typing was done by polymerase chain reaction-sequence specific oligonucleotide probes (PCR-SSP) using kits for generic DNA SSP typing tests of classes I and II, *loci* A, B and DR (One Lambda Inc., CA, USA), according to the manufacturer's instructions.

DNA was amplified using allele-specific primers that accompanied the kits. The regions amplified for A and B *loci* were exons 2 and 3; and for DRB1 *locus*, just exon 3. PCR-SSP molecular assays consisted of a cycle of 2 min and 10 sec at 96°C and of 1 min at 63°C, followed by nine cycles of 10 sec at 96°C and of 1 min at 63°C. Finally, 20 cycles of 10 sec at 96°C, 50 sec at 59°C,

and 30 seconds at 72°C. After the reaction ended, samples were analyzed in agarose gel to confirm *locus*-specific amplification of exons⁽¹⁸⁾.

Statistical analysis

Allele frequencies were obtained by direct count of alleles. For data processing, EPI-INFO™ software was used; see 7.0.9.34 [Centers for Disease Control and Prevention (CDC), Atlanta, EUA]. Statistical analysis consisted of descriptive methods: distribution of absolute and negative frequencies for the qualitative variables, and calculation of average and standard deviation for quantitative variables.

Ethical considerations

This study was approved by the Research Ethics Committee of Hospital Universitário Cassiano Antônio de Moraes, under report no. 1.561.923/2016.

RESULTS

The study involved 208 records of organ donors with brain death from 2011 to 2015. The results achieved in this research are presented in the **Table** and in the **Figure**.

Information regarding sex, age, and ethnic-racial classification are compiled in the Table. Results about sex showed predominance of male donors, representing 63.5% of the records, while female donors accounted for 36.5%. Concerning age, 98 donors were over 40 years, being the majority. Next, comes the age group of 20-39 years, with 72 donors, and then, that of 2-19 years, with 34 donors; four patients' ages were not informed.

Regarding ethnic-racial classification, the obtained results demonstrated predominance of brown color in 94 (45%) donors, followed by those declared white in 59 (28%), black in 28 (14%), yellow in two (1%) and non-informed in 25 (12%).

According to genetic diversity, one notes that about HLA-A, the greatest frequency was that of allele A2 (24.28%), followed by A3 (9.62%) and A68 (7.45%). The least frequent alleles were A43 (0.24%), followed by A36, A69 and A80, each one representing the frequency of 0.48%, and A34 (0.72%) (Figure).

The obtained frequency according to class I-B HLAs were: HLA-B44 (10.34%), being the most frequent in this group, followed by B35 (9.86%) and B7 (8.17%) in the studied population. For the least frequent HLAs, the obtained results were: HLA-B46, B47, B70, B75 and B78, with frequency of 0.24% each. Although there are alleles HLA-B48, B59, B76, B77 and B82, they were not found in the study.

The most frequent class II HLAs (HLA-DR) were DR15 (14.18%), DR13 (13.94%) and DR11 (12.5%); the least frequent, HLA-DR9 (11.68%), DR12 and DR18, with 2.16% each.

TABLE – Sociodemographic profile of deceased donors of solid organs (*n* = 208) in the state of Espírito Santo, Brazil, from 2011 to 2015

| Characteristics | Studied period in years | | | | | Total |
|------------------------------|-------------------------|--------------|--------------|--------------|--------------|--------------|
| | 2011 | 2012 | 2013 | 2014 | 2015 | |
| Sex | <i>n</i> (%) | <i>n</i> (%) | <i>n</i> (%) | <i>n</i> (%) | <i>n</i> (%) | <i>n</i> (%) |
| Female | 10 (13.2) | 19 (25) | 9 (11.8) | 21 (27.6) | 17 (22.4) | 76 (36.5) |
| Male | 21 (15.9) | 30 (22.7) | 29 (22) | 21 (15.9) | 31 (23.5) | 132 (63.5) |
| | | | | | Total | 208 (100) |
| Age | | | | | | |
| 2-19 years | 7 (20.6) | 10 (29.4) | 5 (14.7) | 6 (17.6) | 6 (17.6) | 34 (16.3) |
| 20-39 years | 7 (9.7) | 15 (20.8) | 14 (19.4) | 19 (26.4) | 17 (23.6) | 72 (34.6) |
| > 40 years | 13 (13.3) | 24 (24.5) | 19 (19.4) | 17 (17.3) | 25 (25.5) | 98 (47.1) |
| Not informed | 4 (100) | 0 | 0 | 0 | 0 | 4 (1.9) |
| | | | | | Total | 208 (100) |
| Ethnic-racial classification | | | | | | |
| Black | 3 (10.7) | 6 (21.4) | 7 (25) | 5 (17.9) | 7 (25) | 28 (13.5) |
| White | 7 (11.9) | 19 (32.2) | 12 (20.3) | 16 (27.1) | 5 (8.5) | 59 (28.4) |
| Brown | 8 (8.5) | 19 (20.2) | 18 (19.1) | 21 (22.3) | 28 (29.8) | 94 (45.2) |
| Yellow | 1 (50) | 0 | 1 (50) | 0 | 0 | 2 (1) |
| Not informed | 12 (48) | 5 (20) | 0 | 0 | 8 (32) | 25 (12) |
| | | | | | Total | 208 (100) |

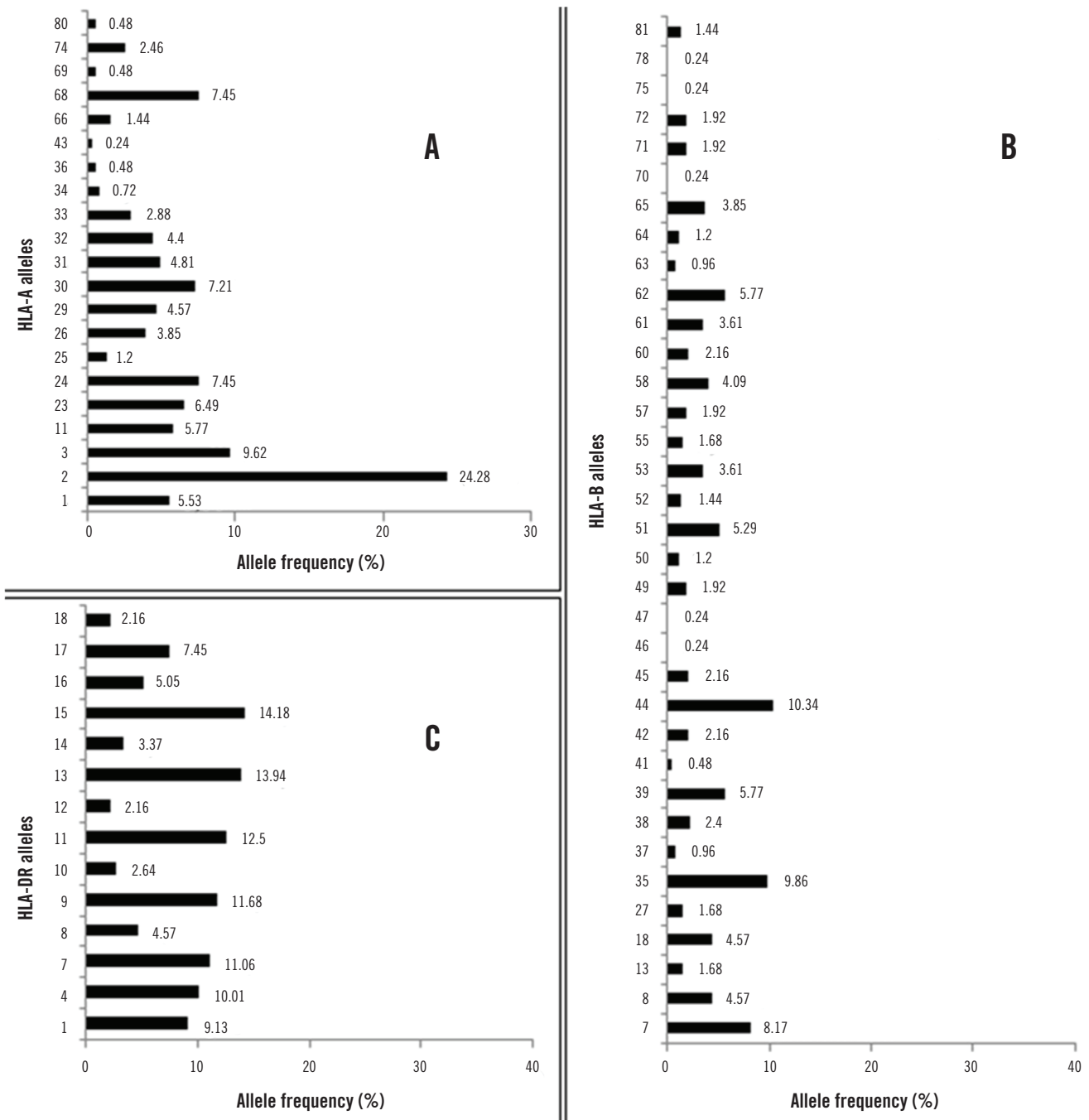


FIGURE – HLA allele frequency (A: HLA-A; B: HLA-B; C: HLA-DR) in deceased donors of solid organs (n = 208) in the state of Espírito Santo, Brazil, from 2011 to 2015

HLA: human leukocyte antigens.

DISCUSSION

In the current study, the HLA alleles most frequently found in the population of deceased donors of solid organs in Espírito

Santo were A2 (24.28%), B44 (10.34%) and DR15 (14.18%); those with the lowest frequency, A43 (0.24%), B46, B47, B70, B75 and B78 (0.24%) and DR9 (1.68%). According to these data, we suppose that the individuals with the alleles most frequently

found can undergo organ transplant with shorter time of waiting in line than those with the least frequent alleles, even if they have been registered for a longer time⁽¹¹⁾. We must highlight the importance of guiding the general population about the possibility of living donation⁽¹⁹⁾ and about organ donation in case of brain death⁽¹¹⁾.

Due to the difficulty of finding statistical studies on HLA screening in cases of solid organ transplantation, a comparison was made between this study and other authors that discussed HLA typing for bone marrow donation. Roque *et al.* (2014)⁽²⁰⁾ brought the information that HLA-A2 (39.2%), HLA-B35 (14.18%) and HLA-DR3 (17.03%) were the alleles most frequently found among bone marrow donors in the Southeast region of Brazil. At a study conducted by Salvadori *et al.* (2014)⁽²¹⁾ in São Paulo, Brazil, the most frequent HLAs among bone marrow donors were HLA-A2 (26.3%), HLA-B35 (12%) and HLA-DR1 (14.9%).

The comparison between the distribution of haplotypes HLA-A and B in two studies in Brazil^(22, 23) revealed that the groups of African origin were similar to the population of Pernambuco; the population of the Northeast of Brazil is predominantly of African origin; the population of Curitiba, predominantly of European origin. This general observation suggests a genetic difference among the ancestry of such populations.

In the present study, 21 HLA-A groups were identified, 35 HLA-B, and 14 HLA-DRB1 allelic groups. In the study conducted by Bortolotto *et al.* (2012)⁽²⁴⁾ in the South of Brazil and by Carvalho *et al.* (2013)⁽²⁵⁾ in the Northeast, almost the same distributions were found: 21 HLA-A groups, 35 HLA-B and 13 HLA-DRB1 allelic groups; and 21 HLA-A groups, 34 HLA-B and 13 HLA-DRB1 allelic groups, respectively.

Comparing the frequencies of alleles and phenotypes of the total sample for the three studied *loci*, the HLA-A*02 allele was the most frequent in this and other studies, regardless the region of the country analyzed^(22, 24-27). In several studies the most common groups for HLA-B alleles were HLA-B*35, -B*44, -B*51, B*40 and -B*15, and in the *locus* DRB1, HLA-DRB1*13, -DRB1*07, -DRB1*05, -DRB1*04 and DRB1*11. The data indicate a contribution of both alleles of European origin⁽²⁸⁻³⁰⁾ and of African origin, such as HLA-B*15⁽²⁴⁾.

Just like Goldberg *et al.* (1998)⁽³¹⁾, we can observe the presence of racially linked haplotypes in individuals diversely classified. They identified an individual classified as white carrying an exclusively African HLA-DR18/DQB1*04 haplotype. The authors explain that this happened several times and

strengthen our notion that racial classification in Brazil is not useful for genetic purposes.

According to the data of the present study, higher prevalence of male donors (63%) was observed. The results are comparable to national data reported by Jornal Brasileiro de Transplantes (JBT), which also presented male predominance (66.7%). These data can be associated with the fact that men are more exposed to risk factors such as traumatic lesions⁽³²⁾.

Predominance of donors aged over 40 years (47%) was observed. This result is similar to national data reported by JBT (2011), which presented donors between 50 and 64 years (31%) and between 35 and 49 years (29%) as the most prevalent age groups⁽³³⁾, as well as data of ethnic-racial classification, which in the present study had higher prevalence of the brown color (45%), agreeing with data published by IBGE⁽³⁴⁾.

The main result of this study was the determination of genetic diversity of HLA (A, B, and DR) alleles from deceased donors of solid organs in the state of Espírito Santo, Brazil. These data have implications for programs of deceased donor organ transplantation. Once one knows the frequency of HLA antigens and the dominant ethnic type in the region, one can predict the waiting time for a recipient in transplant programs, shortening the search for a compatible donor.

CONCLUSION

The profile of deceased solid organ donors found in Espírito Santo, Brazil, in the period 2011-2015, was of brown men aged over 40 years. The most frequent HLA alleles were A2 (24.28%), B44 (10.34%) and DR15 (14.18%); and the least frequent, A43 (0.24%), B46, B47, B70, B75 and B78 (0.24%) and DR9 (1.68%), DR12 and DR18 (2.16%).

Finally, the present study contributed with significant information about the frequency of HLA antigens in deceased organ donors that will be able to contribute to optimize the transplantation process in the country.

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RESUMO

Introdução: A população brasileira possui grande mistura racial, tendo sido originada, principalmente, de caucasóides de origem europeia, africanos e índios. As especificidades do sistema de antígenos leucocitários humanos (HLA) variam entre os diferentes grupos populacionais e étnicos, uma vez que demonstram maior similaridade entre populações com origem comum. **Objetivo:** Determinar a estrutura genética dos alelos HLA de classes I e II em doadores falecidos de órgãos sólidos no Estado do Espírito Santo, Brasil, no período de 2011 a 2015. **Métodos:** O presente estudo foi realizado em 208 indivíduos falecidos doadores de órgãos sólidos, diagnosticados com morte encefálica, no período de 2011 a 2015, no estado do Espírito Santo, Brasil. A tipificação HLA foi realizada pela técnica de reação em cadeia da polimerase-iniciador específico único (PCR-SSP) utilizando kits para teste de tipagem de ácido desoxirribonucleico (DNA) por SSP genérico de classes I e II, loci A, B e DR. **Resultados:** O perfil dos doadores falecidos encontrados no Espírito Santo, Brasil, no período de 2011 a 2015, foi de homens pardos, com faixa etária acima de 40 anos. Os alelos HLA mais frequentes foram A2 (24,28%), B44 (10,34%) e DR15 (14,18%); e os menos frequentes, A43 (0,24%), B46, B47, B70, B75 e B78 (0,24%) e DR9 (1,68%), DR12 e DR18 (2,16%). **Conclusão:** O presente estudo contribuiu com significativas informações acerca da frequência dos antígenos HLA em indivíduos falecidos doadores de órgãos que poderão otimizar o processo de transplantes no país.

Unitermos: doadores de tecidos; alelos; antígenos HLA.

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