

Serological profile of candidates for corneal donation

Perfil sorológico de candidatos a doação de córneas

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ABSTRACT

Objective: The purpose of this study is to map the serological profile of candidates to corneal donation at Irmandade Santa Casa de Misericórdia de Porto Alegre, identifying the percentage of disposal by serology and the marker involved. **Methods:** There have been analysed – retrospectively – the results of serology of all corneal donors, made between the period of 1st January 2006 and 31st December 2012. Data analysed were related to age, gender and the results of serology pertinent to viral markers (HBsAg, anti-HBc, anti-HCV and anti-HIV), these, determined by immunosorbent tests (ELISA). **Results:** In the period of the study, there were 2476 corneal donors at the institution, with a major incidence on the male gender, on an average of 58.7 years old. 23% of retention because of serological unfitness was also identified, that is, 570 samples were non-negative to any of the used tests. The marker anti-HBc was the most prevalent on the studied population, followed by the Hepatitis C virus (HCV) and by the Human Immunodeficiency Virus (HIV). **Conclusion:** From the data found through this study, it is essential to have the participation of an efficient service on the serological evaluation of the candidates to corneal donation, once the security of the receptor must be taken into consideration in a population of donors with 23% of unfitness prevalence, in which the most prevalent marker is the one of Hepatitis B.

Keywords: Serology; Cornea/immunology; Corneal transplantation

RESUMO

Objetivo: O intento deste desígnio é mapear o perfil sorológico dos candidatos a doação de córneas na Irmandade Santa Casa de Misericórdia de Porto Alegre, identificando o percentual de descarte por sorologia e o marcador envolvido. **Métodos:** Foram analisados – retrospectivamente – os resultados da sorologia de todos os doadores de córneas compreendidos entre 01 de janeiro de 2006 a 31 de dezembro de 2012. Foram avaliados os dados de idade, sexo e os resultados da sorologia pertinentes aos marcadores virais (HBsAg, anti-HBc, anti-HCV e anti-HIV) determinados por testes imunoenzimáticos (ELISA). **Resultados:** No período coberto pelo estudo, houve 2476 doadores de córneas na instituição, com maior ocorrência do sexo masculino e média de 58,7 anos de idade. Foram verificados 23,0% de retenção por inaptidão sorológica, ou seja, 570 amostras mostraram-se não-negativas para qualquer dos testes empregados. O marcador anti-HBc foi o mais prevalente na população aferida, seguido pelo vírus da hepatite C (HCV) e pelo vírus da imunodeficiência humana (HIV). **Conclusão:** Diante dos dados encontrados por este estudo, torna-se decisiva a participação de um serviço eficaz no tangente à avaliação sorológica dos candidatos à doação de córnea, uma vez que a segurança do receptor deve ser considerada numa população de doadores com prevalência de retenção por inaptidão sorológica de 23,0%, donde o marcador mais prevalente é o de hepatite B.

Descritores: Sorologia; Córnea/imunologia; Transplante de córnea;

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INTRODUCTION

Corneal diseases are the second cause of reversible blindness around the world and affects a young and active population, leading to an important economic and social problem ⁽¹⁾. The corneal transplant (keratoplasty) has developed fast through the last 10 years ⁽²⁾, and it is considered the most successful procedure among the human tissue transplants ⁽³⁾. Corneal transplantation is indicated to a huge variety of diseases and it provides visual recovering, in an efficient form and low cost, to patients with corneal opacities and irregularities. The success of this surgical procedure has been set due to the fact that the cornea is avascular and there are immunological privileges on this structure. Advances on the surgical technique ⁽⁴⁾, equipment, materials, methods to preserve the donated cornea and postoperative handling have also contributed to the success of the corneal transplants ⁽⁵⁾. The intervention of the eye banks is essential in the search for donors, in the interview with the families, in the quality of processing and distribution of the donated tissues. The quality control of the whole process and distribution of donor cornea begins with the selection of the donors, careful processing using proper techniques of ocular globe enucleation, preservation of the cornea and evaluation of parameters, such as donor serology and endothelial cells counting and the slit lamp evaluation of the donor cornea by an experienced ophthalmologist ⁽⁶⁾.

Because of the increase in the number of corneas donated and kept for longer periods, the careful evaluation becomes an indispensable procedure and of high responsibility by the ocular tissue banks ⁽⁷⁾. Positive serologic testing results are one of the major reasons for discarding donor corneas ⁽⁸⁾. Despite being rare, the potential for the transmission of diseases through organ and tissue grafts exists; even with avascular tissues, as the cornea, have already demonstrated being a way of contamination and transmittion. There are reports of infections caused by bacteria, fungi, viruses and even prions acquired by corneal transplant ⁽⁹⁾. The Brazilian Law does not allow the use of tissues to transplant whose donor present positive serology to hepatitis B, hepatitis C or human immunodeficiency virus (HIV) ⁽⁹⁾ (according to Resolução da Diretoria Colegiada – RDC – number 67). The intention of this study is to map the serological profile of the candidates of corneal donation at Irmandade Santa Casa de Misericórdia de Porto Alegre (ISCMPA), and identify the percentage of disposal by serology and the marker involved.

METHODS

There have been analysed – retrospectively – the results of serology of all corneal donors at ISCMPA between the period of 1st january 2006 and 31st december 2012. Data was obtained through the analysis of the electronic data bank of the institution, Irmandade Santa Casa de Misericórdia de Porto Alegre. All corneas which were captured, evaluated and kept are there in this data bank. There have been evaluated data related to age, gender and the results of serology pertaining to viral markers (HBsAg, anti-HBc, anti-HCV and anti-HIV). The blood samples were obtained according to conditions of accessibility. The blood was kept in a dry and non-preservative tube, centrifuged to 3000 rpm for five minutes and the supernatant serum was used to dosages of immunoenzymatic tests (ELISA). Two tests to detect HIV were used: Genescreen Ultra HIV AgAb Bio-Rad and

Genescreen HIV 1/2 version 2 Bio-Rad). To Hepatitis C, the research of anti-HCV antibodies was made with the kit HCV 3.0 Ortho improved by SAve. To Hepatitis B, anti HBc and HBsAg were measured through the kits anti-HBc Plus Bio-Rad and HBsAg Ultra Bio-Rad, respectively. The results of each serology were classified as fit (negative, according to manufacturer’s criteria) or unfit (inconclusive or positive, according to manufacturer’s criteria). The data is shown in absolute numbers or percentages. No statistical analysis was applied because it is exposed descriptive data.

RESULTS

This study shows that, on average, Irmandade de Santa Casa de Misericórdia de Porto Alegre receives 353 corneas donations a year. During the period of the study, there were 2476 corneal donors at the institution (figure 1), mostly from the male gender (56% male, figure 2) and average of 58,7 years old ($\pm 15,5$, standard deviation) and median and mode of 61 and 62, respectively. Table 1 shows the age distribution of 2182 donors, from whom there had been access to the referred parameter. Twenty-three per cent of retention because of serological unfitness was also identified, that is, 570 samples were non-negative to any of the used tests (figure 3). The marker anti-HBc was the most prevalent on the studied population, followed by the hepatitis C virus (HCV) and by the human immunodeficiency virus (HIV), as shown on table 2.

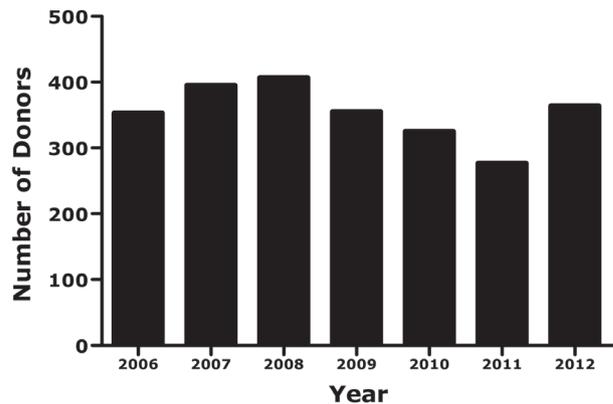


Figure 1: Distribution of the number of candidates to corneal donation stratified per year

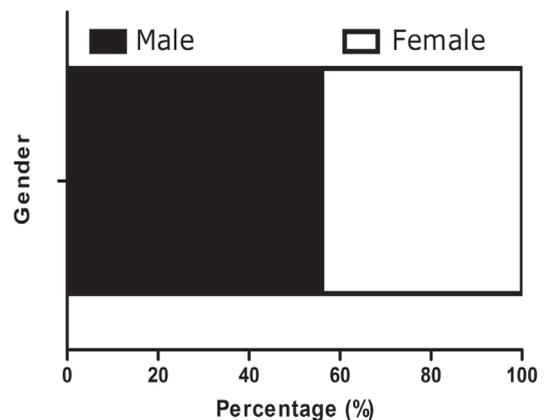


Figure 2: Distribution of corneal donors, showing the percentage of individuals from the male (M) and female (F) gender

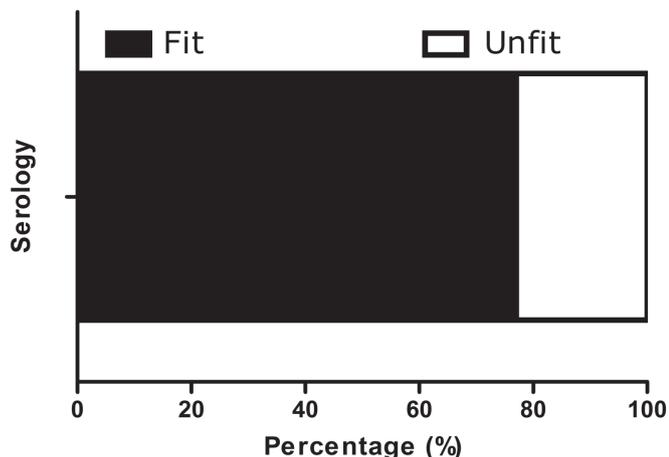


Figure 3: Distribution of the percentual of individuals according to serological fitness

Table 1

Distribution of the percentual of corneal donors according to age range

Age range	(%)
≤ 20	3.16
21 to 30	4.17
31 to 40	4.95
41 to 50	10.72
51 to 60	25.44
61 to 70	26.49
71 to 80	24.66
≥ 81	0.41
Total	100.0

Table 2

Distribution of corneal donors stratified by serology

Test	Frequency	Percentage on the total of donors	Percentage on the total of restraints
HBsAg	9	0.36	1.58
A-HBc	315	12.72	55.26
HCV	113	4.56	19.82
HIV	45	1.82	7.89
A-HBc + HBsAg	27	1.09	4.74
A-HBc + HCV	38	1.53	6.67
A-HBc + HIV	12	0.48	2.11
A-HBc + HCV + HIV	8	0.32	1.40
A-HBc + HBsAg + HIV	2	0.08	0.35
HCV + HIV	1	0.04	0.18
Total	570	23.0	100.0

DISCUSSION

The cornea is the most commonly transplanted tissue in the world ⁽²⁾; however, one of the major restrictions for the increase of transplants is due to the number of donations ⁽¹⁰⁾. In many states of Brazil, the lack of ocular tissues and eye banks which are able to provide corneas in adequate number and quantity to be transplanted is, unfortunately, real ⁽¹¹⁾. However, efforts have been made to change this reality ⁽¹²⁾.

This study shows, according to other authors, that there is a high percentage of donors of the male gender ^(9,13). The minimum and maximum ages of a donor for the usage of the tissue vary according to medical regulations of each eye bank ⁽¹⁾. In this study, the minimum and maximum ages found were of 3 and 84, respectively. The age average found in this study (58.6 years old) supports other studies, which show an age average of 60 ^(1,9), despite other works refer to minimum ages even lower ^(3,13,14). Scientific literature shows that there is no restriction of age to corneas donated and age is not issue to low survival of the graft; what's more, quality in the biomicroscopic and specular microscopic evaluation of the endothelium have to be taken into consideration when using corneas ⁽⁷⁾. However, old age is taken as a limiting factor, once most surgeons prefer not to use corneas from patients over 75 years old. This age is arbitrary, once the age of the donor does not seem to be related to the survival of the graft ⁽¹⁾, though, factors as advanced age range and presence of debilitating diseases tend to coexist and increase the chances of finding a worse evaluation of quality when compared to corneas of young donors and/or trauma deaths ⁽⁷⁾. Studies show that corneas of donors in advanced ages can be perfectly acceptable for transplants, showing that these tissues when coming from older donors (over 80 years old) present a lower chance of failure or post transplant rejection than younger corneas ⁽¹⁵⁾. There are eye banks that stipulate a minimum age for donation, and it can reach 10 years old ^(1,11). Because of this, scientific literature is conflicting and inconclusive about the effect of the age of donors related to the survival of the transplanted cornea ^(16,17).

Viral infections transmitted through transplants have already been reported, once the viruses remain in the tissue after the collection; because of this, the serological screening is an important precondition to the tissue banks ⁽⁸⁾ and the clinic screening does not exclude the undertaking of serological tests. In this study, the corneas to be used in donation, 23% (570) were retained for being considered serologically inappropriate. This percentage is lower than the ones found in other studies, for instance, an eye bank in São Paulo, Brazil, registered a disposal of 33.4% in 2006-2007 ⁽⁹⁾ or the eye bank in Cascavel, a city in the state of Paraná, also in Brazil, reported a serological disposal of 51.8% ⁽¹¹⁾; however, the percentage found in this study is higher than the one found in a study in Minas Gerais, Brazil, where 9% of 1668 corneas were discarded because of positive serology ⁽¹⁸⁾. It is important to mention that the commercial tests are not recommended to serum of corpses; however, there are not specific tests to this end. The corpse sample is usually of low quality. In many cases, the serological tests can show false-positive results in samples post-mortem, which can lead to an unnecessary disposal of the cornea. The time of collection after death and the immediate separation of the serum (centrifugation) are two steps that can affect the quality of the sample ⁽⁸⁾. It is crucial that the multi-disciplined team – militant in the procedures of collection and

storage of ocular tissues – is shrewd to proceed to the blood collection as soon as possible after the death of the donor and make the centrifugation of the collected material right after that. These measures increase serological tests in quality. What's more, it is clear that the combination between the lower period of time from death to the preservation of the material generates better quality of the cornea. Among the explanations of the influence from the time of death and enucleation and/or preservation of the corneal tissue quality are the possible metabolic alterations or even anatomical alterations in the cornea during this process ⁽¹⁵⁾.

The Brazilian Law (RDC 67 from 30th september 2008) demands two serological tests to the detection of hepatitis B (HBsAg and anti-HBc), which exclude donors in case of positivity. In this study, the major part (55.26%) of retentions is due to anti-HBc (table 2) individually, which represents 12.72% from the total of donations. A study made with corneal donors in the region of Cascavel (state of Paraná) revealed disposal because of isolated positivity of anti-HBc of 47.4% on the total of donors. It has already been reported that, in this city, positivity to anti-HBc in donors of blood is significantly higher than in other regions of the same state; however, when the presence of the DNA of hepatitis B virus was detected (a more sensitive and specific test to determine the potential of infectivity of the virus) in donors of blood with positive serology to anti-HBc and negative to HBsAg, there had reached to the result of inexistence of the DNA of the hepatitis B virus in the samples ⁽¹¹⁾. Experts question the adoption of serology anti-HBc to screening corneas ⁽¹⁸⁾ due to its high sensitivity but low specificity ⁽¹¹⁾. The Eye Bank Association of America forebodes only the undertaking of HBsAg as a screening method to hepatitis B, not including in its routine the anti-HBc ⁽¹¹⁾. This idea does not seem reasonable, for most tests used to the serological diagnosis being very sensitive and specific; they cannot detect all cases of hepatitis B. Several studies show that, after undertaking the conventional screening (HBsAg and anti-HBc), when making the research of anti-HBs in the individuals non-reagent to HBsAg, but reagent to anti-HBc, 10 to 34% will be also non-reagent to anti-HBs, being classified as anti-HBc isolated ⁽¹⁹⁾. The presence of this profile is consistent with (a) old infection with low levels of anti-HBs, (b) period of immunological window, (c) false positive reaction and (d) HBsAg chronic carrier non-detectable. Through tests of amplification of nucleic acids, it was proved the presence of the DNA of the hepatitis B virus in 1.34% of donors HBsAg negative with anti-HBc positive ⁽¹⁹⁾. The detection of cases with anti-HBc isolated can be avoiding not only the transmission of wild strain (not detected due to the low viral load present in individuals with non-detectable levels of HBsAg) but also the transmission of mutant strains ⁽¹⁹⁾. Nowadays, the non-detection of HBsAg marker due to the appearance of these mutant strains has been cause of concern. The analytic sensitivity of tests to the detection of HBsAg may rely on the genotype or subtype of the hepatitis B virus ⁽¹⁹⁾.

In this study, the presence of anti-HBc together with HBsAg was found in 4.74% of unable donors, reaching 1.09% of the total of donations. This rate is similar to the one in the region of Cascavel, which shows 1.5% of retention to this association of markers ⁽¹¹⁾. This profile is consistent with the acute phase or the chronic carry of hepatitis B ⁽²⁰⁾.

Also, in this study there is a report on table 2 about the prevalence of 4.56% to HCV and 1.82% to HIV. These levels are

superior to the ones found in eye banks of São Paulo ⁽⁹⁾ and Cascavel ⁽¹¹⁾. The presence of anti-HBc together with HCV was found in 38 candidates to donation, prevailing 1.53% (6.67% among the retained ones). This situation is common, and it can represent several other situations, among them, hidden hepatitis B in patients with chronic hepatitis C ^(21,22).

The usage of nucleic acid tests to confirm (or not) positive results is foreboded once these tests would really detect the existence of infection, for antibodies detected through traditional serological methods could persist for longer time after active infection has stopped; however, one of the limiting factors to serological screening with acid nucleic tests are the costs ⁽⁹⁾. Tissue Banks must evaluate the usage of this kind of test because they reduce the risk of viral infections caused by grafts ⁽⁸⁾. This ideal situation is still not tangible to our reality, because of the excessive burden of the process.

The plurality of professionals involved in a transplant of cornea is imperative facing the extensive work done in this process. It is essential, though, that the teams are aligned with the development of the work. It is essential to have the participation of an efficient service on the serological evaluation of the candidates to corneal donation, once the security of the receptor must be taken into consideration in a population of donors with high unfitness prevalence. In this context, the adoption of nucleic acid tests will enhance quality to the process in a near future.

Works like this one are of elementary importance because they bring – to the scientific community – the profile of the corneal tissues donated, and from these data, they enable institutions to take measures in relation to implementing services of eye banks based on the knowledge they have about the target public. In our study, we show retention of 23% for serological unfitness. This index is considered high if compared to serological retention of blood donation, for instance. This fact occurs because there is no clinic retention to candidates of corneal donation, once the personal interview is impossible to be made, as the donors are corpses. The most prevalent marker was referent to Hepatitis B, followed by HCV and HIV. This study confirms the validity and the importance of serological tests so to exclude positive serological corneas to prevent infections, which could be transmitted to eventual cornea receptors

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