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Original Article

Prevalence of microbiological markers in bone tissue from live and cadaver donors in the musculoskeletal tissue bank of Passo Fundo☆,☆☆

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ABSTRACT

Objective: To conduct an epidemiological analysis on the main microbiological markers in bone tissue that was processed at the musculoskeletal tissue bank of Hospital São Vicente de Paulo, in Passo Fundo, between August 2007 and October 2011.
Methods: Between August 2007 and October 2011, 202 musculoskeletal tissue samples were collected for the tissue bank. Among these, 159 samples were from living donor patients and 43 were from cadaver donors. The following serological tests were requested: hepatitis B, hepatitis C, syphilis, cytomegalovirus, Chagas disease, toxoplasmosis, HIV and HTLV.
Results: Among the 159 living donors, 103 (64.75%) were men and 56 (35.25%) were women. The patients' mean age was 59.35 ± 8.87 years. Out of this total, 76 tissue samples (47.8%) from donors were rejected. There was no difference in the number of rejections in relation to sex ($p = 0.135$) or age ($p = 0.523$). The main cause of rejection was serologically positive findings for the hepatitis B virus, which was responsible for 48 rejections (63.15%). Among the 43 cadaver donors, the mean age was 37.84 ± 10.32 years. Of these, 27 (62.8%) were men and 16 (37.2%) were women. Six of the samples collected from cadaver donors were rejected (13.9%), and the main cause of rejection was serologically positive findings for the hepatitis C virus, which was responsible for three cases (50%). There was no significant difference in the number of rejections in relation to sex ($p = 0.21$) or age ($p = 0.252$).
Conclusion: There were a greater number of rejections of tissues from living donors (47.8%) than from cadaver donors (13.9%). Among the living donors, the main cause of rejection was the presence of serologically positive findings of the hepatitis B virus, while among the cadaver donors, it was due to the hepatitis C virus.

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Prevalência de marcadores microbiológicos em tecido ósseo de doadores e cadáveres do Banco de Tecidos Musculoesqueléticos de Passo Fundo

Palavras-chave:
Bancos de ossos
Transplante homólogo/efeito adverso
Doenças virais
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RESUMO

Objetivo: fazer uma análise epidemiológica dos principais marcadores microbiólogicos dos tecidos ósseos processados de agosto de 2007 a outubro de 2011 no Banco de Tecidos Musculoesqueléticos do Hospital São Vicente de Paulo de Passo Fundo.

Métodos: foram feitas 202 captações de tecidos musculoesqueléticos para o Banco de Tecidos. Desse total, 159 foram de doadores e 43 de cadáveres. Foram solicitados testes sorológicos para hepatite B, hepatite C, sífilis, citomegalovírus, doença de Chagas, toxoplasmose, HIV e HTLV.

Resultados: dos 159 doadores, 103 (64,75%) eram do sexo masculino e 56 (35,25%) do feminino. A idade média foi de 59,35 ± 8,87 anos. Foram descartados 76 (47,8%) tecidos de doadores. Não houve diferença significativa no número de descartes em relação a sexo (p = 0,135) ou idade (p = 523). A principal causa de descarte foi a sorologia positiva para o vírus da hepatite B, responsável por 48 (63,15%) descartes. Já entre os 43 cadáveres, a média de idade foi de 37,84 ± 10,32 anos. Desses, 27 (62,8%) eram do sexo masculino e 16 (37,2%) do feminino. Foram descartados seis (13,9%) cadáveres. A principal causa de descarte foi a sorologia positiva para o vírus da hepatite C, responsável por três (50%) casos. Não houve diferença significativa no número de descartes em relação a sexo (p = 0,21) ou idade (p = 252).

Conclusão: houve um número maior de descarte de tecidos de doadores (47,8%) em comparação com os cadáveres (13,9%). Nos doadores, a principal causa de descarte foi a presença de sorologia positiva para o vírus da hepatite B; nos cadáveres, para o vírus da hepatite C.

Introduction

Musculoskeletal tissue banks have presented significant progress over the recent years and have become a tool of immense importance for undertaking orthopedic surgical procedures of greater complexity, through providing a plentiful and safe supply of bone, fascial, chondral and ligament material for grafts.[1,2] Orthopedic surgeons' concern regarding disease transmission through bone grafts is diminishing because of the rigorous selection criteria, high sensitivity of serological tests and serial examinations performed during harvesting, storage, processing and sterilization of these grafts.[3–8] The musculoskeletal tissue bank at Hospital São Vicente de Paulo (HSVP), in Passo Fundo, was founded in 1982, with adaptation to new legislation in 1995. Its aim is to adequately store bone and tendon tissue and thus benefit patient treatment in the orthopedic-traumatological and dental fields.

The present study aimed to retrospectively analyze the prevalence of the main microbiological markers in bone tissues in the musculoskeletal tissue bank at Hospital São Vicente de Paulo, in Passo Fundo, and the possible causes of rejection of material.

Materials and methods

Between August 2007 and October 2011, at Hospital São Vicente de Paulo, 202 musculoskeletal tissue-harvesting procedures were performed to acquire material for the tissue bank. Out of this total, 159 were from donors and 43 were from cadavers.

The donors underwent orthopedic surgical procedures for total hip arthroplasty in which the femoral head was removed. A questionnaire was applied and a detailed history of the donors' previous medical history was obtained. In addition, preoperative laboratory tests were run in order to detect infectious diseases. The following serological tests were requested: hepatitis B (anti-HBsAg and anti-total HBc), hepatitis C (anti-HCV), syphilis (hemagglutination of *Treponema pallidum*, VDRL), cytomegalovirus (anti-CMV IgG and IgM), Chagas disease (anti-*T. cruzi*), toxoplasmosis (anti-*Toxoplasma* IgG and IgM), HIV (anti-HIV 1 and 2) and HTLV (anti-HTLV 1 and 2). The serological tests on the donors were repeated six months after the harvesting procedure. The tissues were not released for use until after the final results from the above tests had been obtained. Tissue donated by patients whose serological tests for infectious diseases in any of the samples were positive, or who fell within the exclusion criteria of the protocol, was discarded (Table 1).

Harvesting of bone tissue from the femoral head was done in a surgical environment, by a medical team specializing in primary hip arthroplasty procedures. The tissue removed was subjected to two sample collections for culturing and microbiological tests, over the entire length of the bone tissue. One sample was for culturing fungi and the other was for culturing bacteria. After collection of material for microbiological tests, the tissue was washed in physiological solution using 1 g of cefazolin (500 mL of 0.9% physiological serum for 1 g of cefazolin). All the soft tissues, muscle insertions and periosteum were then cleaned so that the material could be packed in tripled-sealed sterile packaging and were sent to the tissue bank. The tissues selected were stored in sterile receptacles

Table 1 – Exclusion criteria for donor tissue.

Malignant neoplasia	Treatment with growth hormones
AIDS	Jaundice of non-obstructive cause
Degenerative neurological diseases	Individuals who underwent acupuncture, tattooing or application of permanent makeup over the 12 months prior to the donation
Major burns	Use of toxic substances
Creutzfeldt-Jakob disease	Treatment with radiotherapy or chemotherapy (except for treatment of benign pathological conditions)
Diseases of unknown etiology	Homosexual habits
Malaria	Sex in exchange for money
Systemic bacterial or fungal infection	Heterosexuals with multiple sexual partners
Individuals undergoing dialysis or the sexual partners of such individuals	Accidents with suspected blood; unconfirmed examinations for HIV, hepatitis B or C
Organ or tissue transplantation within 12 months of the donation	Imprisoned individuals or individuals held in prison institutions for more than 24 h
Active tuberculosis	Individuals who have had sexual intercourse with people who have been tested positive for HIV and hepatitis B and C

that were placed in a freezer set at −80 °C, with storage validity for up to five years after harvesting, in accordance with the ideal temperature conditions and the standards established by the National Sanitary Surveillance Agency (ANVISA) and the American Association of Tissue Banks (AATB). All the results from serological tests were documented and filed in the donors' records, and were cautiously verified by the biologists and nurses responsible for the bone bank.

The cadavers were carefully selected and evaluated in accordance with the standards and guidelines established by AATB and ANVISA. The potential musculoskeletal tissue donors were brain-dead patients aged 18–70 years whose families consented to organ donation. The material was removed in a surgical environment by a specialized team consisting of four orthopedists, a biologist, a nurse and a nursing technician. After collection, the material was subjected to the same selection, examination and storage criteria as the material collected from donors.

The data were analyzed using the SPSS 13.0 software. The categorical variables were described as percentages and the continuous variables, as means and standard deviations. The chi-square test was used for categorical variables and Student's *t* test was used for continuous variables.

Results

Out of the 159 femoral head donors between August 2007 and October 2011, 103 (64.75%) were men and 56 (35.25%) were women. The patients' mean age was 59.35 ± 8.87 years: 61.0 ± 8.8 years for the women and 58.4 ± 8.8 years for the men. Tissue from 76 donors (47.8%) was discarded: 32 women (42%) and 44 men (58%). There was no significant difference in the number of rejections in relation to sex ($p = 0.135$) or age ($p = 0.523$), among the grafts coming from donors (Fig. 1).

Table 2 – Causes of rejection of donor tissue.

Microbiological and serological tests	Number of donors with positive tests who were rejected
HBV (anti-HBsAg and anti-total HBc)	48
HCV (anti-HCV)	2
Syphilis (hemagglutination of *T. pallidum*, VDRL)	1
CMV (anti-CMV IgG and IgM)	4
Chagas disease (anti-*T. cruzi*)	0
Toxoplasmosis (anti-*Toxoplasma* IgG and IgM)	2
HIV (anti-HIV 1 and 2)	0
HTLV (anti-HTLV 1 and 2)	1
Positive bacterial cultures	2
Positive fungal cultures	3
Other exclusion criteria	13
Total	76

The main cause of rejection was a positive serological test for the hepatitis B virus, which accounted for 48 rejections (63.15%). Positive serological tests for cytomegalovirus accounted for four rejections (5.2%), followed by positive serological tests for the hepatitis C virus, with two rejections (2.6%); toxoplasmosis, with two (2.6%); HTLV, with one (1.3%); and syphilis, with one (1.3%). Bacterial contamination accounted for two rejections (2.6%); *Streptococcus* sp. was found in both of these cultures. Contamination by filamentous fungi accounted for three rejections (3.9%). The other rejections were for other reasons, in accordance with the exclusion criteria. None of the material in the present study was found to be serologically positive for HIV or anti-*T. cruzi* (Table 2).

Among the 43 cadavers, 27 (62.8%) were men and 16 (37.2%) were women. The mean age was 37.84 ± 10.32 years. Tissues from six cadavers were discarded (13.9%). There was no significant difference in the number of rejections in relation to sex ($p = 0.21$) or age ($p = 0.252$) (Fig. 2).

The main cause of rejection among the cadavers was a positive serological test for the hepatitis C virus, which accounted for three rejections (50%). A positive serological test for toxoplasmosis was responsible for one rejection (16.6%). The others were excluded for other reasons, in accordance with the exclusion criteria (Table 3).

Table 3 – Causes of rejection of cadaver tissue.

Microbiological tests	Number of rejections among cadavers
Hepatitis C (anti-HCV)	3
Toxoplasmosis (anti-*Toxoplasma* IgG and IgM)	1
Insufficient sample for analysis	1
Contamination during harvesting of material	1
Total	6

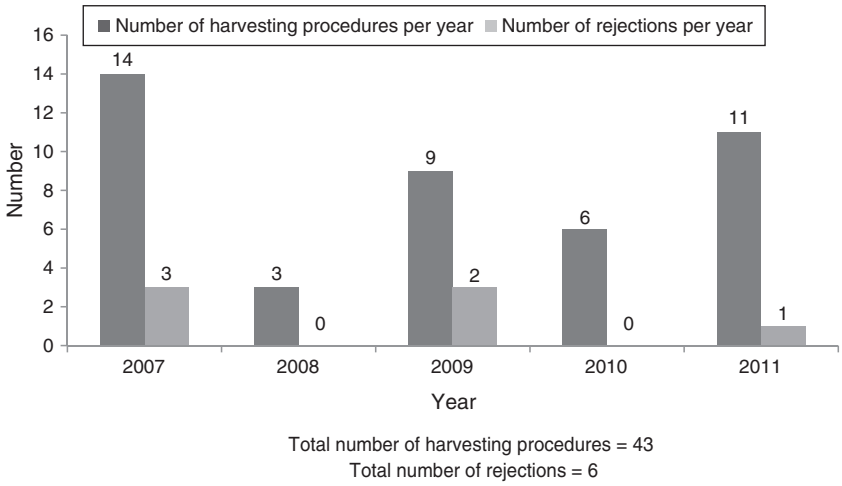

Fig. 1 – Relationship between numbers of harvesting procedures among donors and rejections performed between 2007 and 2011.

Discussion

Bone grafts in orthopedic surgery are becoming more and more frequently used, in tumor resection procedures, spinal surgery, revision on hip and knee prostheses and procedures dealing with sequelae of trauma.[9–13] Use of homologous bone grafts from tissue banks has been shown to be advantageous in many respects: there is no need for a secondary surgical access to remove tissue and it is possible to obtain a large quantity of material and a variety of anatomical forms.[14,15] Other advantages include their biocompatibility, low immunogenicity and modest operational cost. Every year, around 150,000 musculoskeletal allografts (including bone, tendon and cartilage) are used by orthopedic surgeons in the United States.[2] Differing from other transplants, the bone rejection rate is minimal and patients do not require immunosuppressant medications.

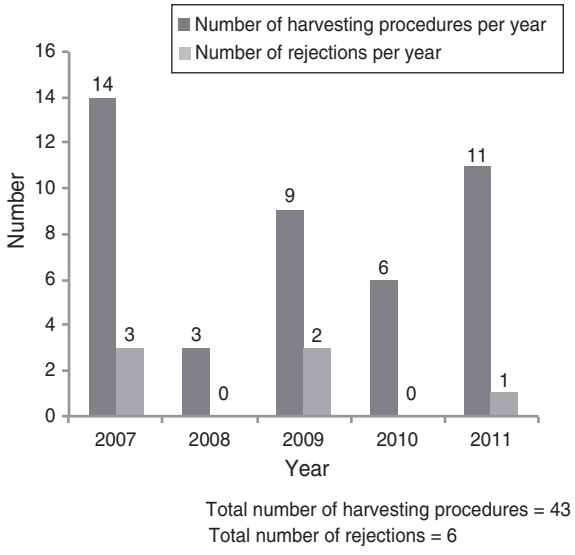

Fig. 2 – Relationship between numbers of harvesting procedures among cadavers and rejections performed between 2007 and 2011.

Contamination of the bone graft material is not a rare event, and the incidence of rejection reported in the medical literature ranges from 17% to 50%.[9–13] This variation in the bone tissue rejection rate is related to the incidence of infectious-contagious diseases in the region and also to the care and protocols established for harvesting and handling tissues.[12–15] Because of the rigorous protocol followed in our tissue bank, our rejection rate after harvesting the bone tissue has so far been 40.5%. According to records in the current literature, the incidence of contamination in bone graft material coming from cadavers is higher than the rate among donors.[10–13] The rejection rate for musculoskeletal tissues harvested from cadavers is related to the length of time between death and tissue removal.[13]

Although the risk of virus transmission by means of musculoskeletal allografts is considered to be low, the greatest disadvantage in using bone allografts is the possibility of transmitting diseases. Tomford[7] reported four cases of HIV transmission and three of hepatitis C transmission after bone graft donation in the United States, up to 1995. After the confirmations of these cases, the AATB formulated rigorous protocols to be followed by tissue banks and care to be taken in choosing donors and harvesting, storing and processing tissues. Since then, according to the AATB, and in conformity with the guidance and standards established, the chance of HIV transmission in bone transplants in the United States has been one in 25 million.[4] In Brazil, no similar statistical data are yet available. In our tissue bank, there have not been any records of disease transmission to bone graft recipients so far.

Recent studies have noted that frozen bone grafts that had been properly stored, and for which the criteria for protection against contaminations had been followed, did not present alterations to their structure and maintained their qualities for an indeterminate length of storage.[3] Nonetheless, in our service, we have determined that a period of five years, counted from the date of harvesting, is the limit for using stored grafts, in accordance with the standards recommended by ANVISA and AATB.

Frozen bone grafts obtained under aseptic conditions function as the basis for bone neoformation and act more in an osteoconductive than an osteoinductive manner. In our tissue bank, we do not use sterilization by means of radiation. The tissues are frozen while fresh and are processed in a clean and sterile area (air class 100 by means of laminar flow in the processing area). For this reason, the protocols and care in harvesting the material are followed rigorously. If there is any type of contamination, the tissues are discarded. In this manner, the recipient's safety is assured and the risks of disease transmission are reduced to insignificant levels.

At Hospital São Vicente de Paulo, the team led by Dr. Milton V. Roos performed the first bone transplantation 30 years ago, and the first transplantation of the proximal third of the femur 25 years ago. Our hospital has become consolidated as the institution with the oldest musculoskeletal tissue bank in continuous operation in Brazil. At the time of its founding, there were no rules for transplants. In 1997, through Law No. 9434, of February 4, 1997, organ transplantation services were officially created. On September 20, 2002, Ordinance 1686 of the Ministry of Health determined standards for musculoskeletal tissue banks to function throughout the country. After adaptation to the new requirement, through Ordinance No. 556, of October 5, 2005, from the Ministry of Health, the musculoskeletal tissue bank of HSVP received regulatory authorization. From that time until the second semester of 2011, 202 harvesting procedures were performed and 2650 tissue grafts were applied to 1869 recipients.

Conclusion

Grafting of bone material coming from tissue banks has been shown to be safe and is increasingly used in high-complexity orthopedic procedures. There is a risk of transmission of infectious-contagious diseases, but this potential risk of contamination of the recipient can be reduced if appropriately rigorous microbiological monitoring is undertaken.

In the present study, there was a higher rejection rate for tissue from donors (47.8%) than for tissue from cadavers (13.9%). This was probably due to the greater age of the donors than that of the cadavers and, consequently, the greater length of exposure to infectious-contagious diseases.

Conflicts of interest

The authors declare no conflicts of interest.

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