Limbal transplantation at a tertiary hospital in Brazil: a retrospective study

Transplante de limbo em hospital terciário no Brasil: estudo retrospectivo

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ABSTRACT

Purpose: Epidemiological analysis of limbal transplantation surgeries performed in the Ophthalmologic Hospital of Sorocaba. Methods: Retrospective medical records review of 30 patients who underwent limbal stem cell transplants between January 2003 and March 2008. Cases involving conjunctival limbal autograft were classified as group I, and those involving conjunctival limbal allograft as group II. Results: Two patients were excluded due to incomplete data during postoperative follow-up. Of the total sample of 28 patients, 53.6% constituted group I, whereas 46.4% were included in group II. Males were predominant (67.9%), and right eyes were the most prevalent (67.9%). The mean age was 40.3 years. Unilateral cases accounted for 60.7%. The most frequent pathology causing limbal system failure was chemical burns (53%). The mean length of time from diagnosis to surgery was 11.18 years. The limbal graft and amniotic membrane were associated in 75% of all cases, and tarsorrhaphy in 57.1%. The average follow-up period was 24.84 months. The uncorrected visual acuity improved in 38% of the cases, was unchanged in 28.5%, and deteriorated in 33.3%. There was no persistent epithelial defect in 75% of the patients. The conjunctivalization rate was similar between the groups (53.3% and 58.3%, respectively). The transparency improved in only 38.4% of the cases, and 28.5% of the surgeries performed were successful. The most prevalent complication was persistent epithelial defect, which occurred in 25% of the patients, followed by corneal melting in 14.2%. Other complications observed included infectious ulcers, limbal graft necrosis or ischemia, perforation, and descemetocele. Conclusion: Chemical burns remain the main cause of limbal stem cell deficiency. In these cases, limbal transplantation is the standard procedure to restore the ocular surface even though the success rate is low. Keywords: Limbus corneae; Transplantation, autologous; Transplantation, homologous; Stem cells; Conjunctival diseases; Burns, chemical

INTRODUCTION

The epithelium of the limbus and cornea ensure the functional and anatomical stability of the normal ocular surface of the human eye(1). Ridges in the conjunctival epithelium toward the peripheral corneal epithelium can be noted in the corneoscleral limbus, which were first described in 1921, and are now known as the palisades of Vogt(2). Fifty years later, Davanger and Evensen suggested that these ridges harbor corneal epithelial precursor cells(3).

In 1983, Thoft and Friend put forth a theory, known as XYZ, which determined the role of limbal stem cells in corneal epithelium renewal and maintenance(4). The corneal epithelium is in a state of constant renewal to ensure ocular surface maintenance, and this homeostasis depends on limbal stem cells.

Some situations, including thermal and chemical burns, autoimmune diseases, limbal failure secondary to contact lenses use, cryotherapy, or radiation, may alter or reduce the ability of the limbus to serve as a source of stem cells. In the wake of limbal stem cell deficiency, chronic epithelial defect, recurrent corneal erosion, neovascularization, chronic inflammation, fibrosis, and squamous metaplasia may occur(5).

Currently, the most common surgical technique to restore normal limbal physiology is limbal stem cell transplantation (LSCT)(6). The donor tissue can be taken from the same eye (ipsilateral conjunctival limbal autograft [CLAU]), the contralateral eye (contralateral CLAU), from a human leukocyte antigen (HLA)-matched related (HLA-1) and unrelated (HLA-2) limbal donor tissue. The success rate of LSCT is reported to be 28% in the first year(7).

RESUMO

Objetivo: Análise epidemiológica dos transplantes de limbo realizados no Hospital Oftalmológico de Sorocaba. Método: Foi realizada uma análise retrospectiva através de revisão de prontuário de trinta casos de transplante de limbo realizados no período de janeiro de 2003 a março de 2008. Casos de transplante de limbo autólogo foram classificados como grupo I e alógenos como grupo II. Resultados: Dois pacientes foram excluídos da análise por insuficiência de dados nos registros. Da amostra completa de 28 pacientes, 53,6% constituíram o grupo I enquanto 46,4% o grupo II. O olho direito (68%) e o sexo masculino (68%) foram mais frequentes, com uma média de idade de 40,3 anos. Casos unilaterais contabilizaram 60,7%. A patologia de base causadora da deficiência limbal mais prevalente foi a queimadura química (53%). A média do tempo de doença até a cirurgia foi de 11,18 anos. Na maioria dos casos o transplante foi associado à membrana amniótica (75%) e tarsorrifa (57%). O tempo médio de seguimento foi de 24,84 meses. Foi observado melhora da acuidade visual não corrigida em 38% dos casos enquanto 28,5% permaneceram inalterados e 33,3% pioraram. Evolução sem defeito epitelial persistente ocorreu em 75% dos pacientes. A taxa de conjuntivalização foi semelhante nos 2 grupos (53,3% e 58,3%). Em apenas 38% dos casos houve melhora da transparência. A taxa de sucesso foi de cerca de 28%. A complicação mais prevalente foi defeito epitelial persistente (25%) seguido de melugão (14,2%). Outras complicações observadas foram úlceras infecciosas, necrose ou isquemia do conjuntivo, perifissura e descemetocele. Conclusões: A queimadura química permanece como principal patologia causadora de deficiência limbal. Nestes casos o transplante de limbo é atualmente a técnica de eleição para restauração da superfície ocular, porém com baixa taxa de sucesso. Descritores: Limbo da córnea; Transplante autólogo; Transplante homólogo; Células-tronco; Doenças da túnica conjuntiva; Queimaduras químicas.

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depending on the severity and unilaterality or bilaterality of the clinical condition.

The purpose of this study is to report on the experience of the Ophthalmologic Hospital of Sorocaba (HOS), a tertiary teaching hospital, in performing these procedures, as well as the success and failure rates and associated complications.

METHODS

This is a retrospective study conducted between January 2003 and March 2008. During this period, 30 LSCT procedures were performed.

This study was approved by the Research Ethics Committee of the HOS, and adhered to the recommendations of the Declaration of Helsinki.

Any patient undergoing LSCT during the time of the study, regardless of the cause of limbal failure, patient age, or severity, was included in the study. Patients who did not undergo sufficient postoperative follow-up to determine the failure or success of the procedure were excluded. Two patients were excluded. Cases involving autologous grafts were classified as group I, and those involving allogenic grafts as group II.

A fellow surgeon assisted by an experienced cornea surgeon performed all the surgical procedures. All surgeries were performed under peribulbar anesthesia with 2% lidocaine. In group I, two fragments of the limbus and bulbar conjunctiva were obtained from the upper and lower regions of the healthy contralateral eye (CLAU). In group II, the grafts were obtained either from a living-related donor (lr-CLAL) after human leukocyte antigen I and II typing and cross-matching or from cadaver donor tissue (c-CLAL). In the recipient eye, a complete conjunctival peritomy was performed, followed by a lamellar keratectomy to remove fibrous pannus from the cornea. The limbal fragments were attached to the upper and lower limbus using a 10-0 monofilament nylon suture (Figure 1). In cases in which the amniotic membrane (AM) was used, it was placed either over or under the graft, or both over and under (“sandwich” technique).

The postoperative regimen was not standardized but all cases included corticosteroids and antibiotic eye drops (minimum of 4 weeks and 2 weeks, respectively) and preservative-free artificial tears. Systemic immunosuppression was implemented as a standard procedure in cases of c-CLAL and in lr-CLAL in which histocompatibility was not 100%. Systemic immunosuppression regimen and management were determined by a referred nephrologist and were not available in the medical records.

The data analyzed in this study included: gender, age, operated eye, condition causing limbal failure, time elapsed from the onset of the disease until surgery, unilaterality or bilaterality, autologous or allogenic transplantation, kinship of the donor in the case of allogenic transplantation, limbal extension, use of AM and positioning of its epithelial face, combination with tarsorrhaphy, duration of postoperative follow-up, probable cause of failure, uncorrected visual acuity before and after surgery, persistent epithelial defect or con-

Figure 1. Surgical steps in a case of homologous limbal stem cell transplantation. A) preoperative photograph of a case of unilateral limbal stem cell deficiency; B) removal of fibrovascular tissue from corneal and scleral surfaces; C) underlying corneal and scleral bed preparation; D) autologous limbal tissue dissected from contralateral healthy eye at 6 o’clock position; E) autologous limbal tissue ready for transplantation; F) amniotic membrane transplantation; G) autologous limbal tissue sutured in place at 6 o’clock position under amniotic membrane transplantation (epithelial face down); H) immediate postoperative photograph; I) postoperative photograph on the seventh day.
junctivalization of the cornea after surgery, improvement of corneal transparency, and postoperative complications.

In cases where persistent epithelial defect or conjunctivalization was not detected during the postoperative follow-up, the intervention was defined as a surgical success. All biomicroscopic findings reported that suggested failure, such as ischemia, melting, presumed rejection, necrosis, epithelial defect, or conjunctivalization were defined as failures.

Kaplan-Meier survival analysis was performed to evaluate conjunctival limbal graft survival.

RESULTS

The preoperative evaluation was performed for all 28 patients, from groups I and II, included in the study (Table 1).

Of the total sample of 28 patients, 53.6% (n=15) underwent a CLAU and constituted group I, whereas 46.4% (n=13) underwent a CLAL and constituted group II.

Males were predominant in both groups (Table 1), and consequently, constituted the majority of the overall population studied: 67.9% (n=19). Right eyes were the most prevalent in the overall sample, at 67.9% (n=19), with 73.3% (n=11) in group I and 61.5% (n=8) in group II.

In relation to patient age at the time of surgical intervention, the mean age was 40.33 years (5-82 years). A higher mean age was found in group I, as seen in Table 1.

The groups differed with respect to bilaterality. Unilateral cases predominated in group I and bilateral cases predominated in group II as shown in Table 1. In the overall analysis, unilateral cases accounted for 60.7% (n=17) and bilateral for 39.2% (n=11).

The most frequent pathology causing limbal stem cell deficiency among the entire study population was chemical burns (53%), followed by thermal burns (20.0%, n=3) and failure secondary to multiple surgical procedures (13.3%, n=2) in group I, and by Stevens-Johnson syndrome (23.0%, n=3), and atrophy, aniridia, and brachytherapy (each corresponding to 7.6%, n=1) in group II.

The duration of time of disease progression from diagnosis until surgery ranged from 1 month to 52 years, with an average of 11.18 years (Table 1).

The extension of donor limbal tissue transplanted was evaluated and the results are shown in Table 1. The limbal tissue extension most used in the study was 120°, at 31.2% (n=5).

The groups were evaluated regarding the use of AM during surgery, as well as the positioning of its epithelial face. AM was used in the majority of the patients in the study: 75.0% (n=21); with 80.0% (n=8) in group I and 61.5% (n=8) in group II.

In relation to the extension of donor tissue transplanted, the mean was 120°, at 31.2% (n=5). The groups differed with respect to positioning: upward was used in three cases and downward in only one case.

In cases where persistent epithelial defect or conjunctivalization was not detected during the postoperative follow-up, the intervention was defined as a surgical success. All biomicroscopic findings reported that suggested failure, such as ischemia, melting, presumed rejection, necrosis, epithelial defect, or conjunctivalization were defined as failures.

Kaplan-Meier survival analysis was performed to evaluate conjunctival limbal graft survival.

<table>
<thead>
<tr>
<th>Epidemiological characteristics</th>
<th>Group I (n=15)</th>
<th>Group II (n=13)</th>
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<tr>
<td>Autologous transplantation (CLAU)</td>
<td>48.83 (29-82)</td>
<td>31.83 (5-63)</td>
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<td>Sex (male/female)</td>
<td>11/4</td>
<td>8/5</td>
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<tr>
<td>Bilaterality</td>
<td>2 (13.3%)</td>
<td>9 (69.2%)</td>
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<td>Mean time until surgery (years)</td>
<td>9.61</td>
<td>12.62</td>
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<tr>
<td>Limbal extension used</td>
<td>≤120° (100%)</td>
<td>&gt;120° (67.5%)</td>
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<tr>
<td>Use of amniotic membrane</td>
<td>12 (80.0%)</td>
<td>9 (69.2%)</td>
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<tr>
<td>Tarsorrhaphy</td>
<td>6 (40.0%)</td>
<td>10 (76.9%)</td>
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<tr>
<td>Mean postoperative follow-up (months)</td>
<td>18.36</td>
<td>35.04</td>
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</table>

DISCUSSION

Many studies have already reported that limbal stem cells are the source of epithelial renewal and regeneration under normal or injury conditions.

The replacement of limbal stem cells through LSCT, is one of the most effective methods for treating limbal stem cell deficiency. The technique is chosen depending on the extent and severity of the injury.
grafts (group II).

Kaplan-Meier survival curve of autologous grafts (group I) and allogenic grafts (group II). Since the main cause of limbal stem cell deficiency in this study was chemical injury, we believe that the use of systemic immunosuppression is considered.

In this study, the male sex (67.9%) and right eye were more commonly affected. A previous small series has also reported a higher prevalence among males. Since the main cause of limbal stem cell deficiency in this study was chemical injury, we believe that the higher incidence in males may be related to greater occupational exposure in males.

The distribution of patients in groups I and II took into account the severity and unilaterality or bilaterality. This fact limits comparisons between the two groups insofar as the success and failure rates of the treatments applied, since it involved patients with different clinical profiles who also underwent distinct surgical procedures. Success rate analysis should also take into consideration the technical inexperience of surgeons who were still learning.

Only 13.3% (n=2) of the 15 patients in group I had bilateral disease, whereas 69.2% of the cases in group II had bilateral involvement (n=9). In the two cases where there was bilateral disease and CLAU was performed, the patients had partial and localized limbal failure in the least affected eye caused by chemical burns. In group II, four patients had unilateral disease (30.8%), and despite this, underwent CLAL. Three of them had previously undergone unsuccessful CLAU and one had a contralateral eye in phtisis bulbi.

The literature on the subject indicates that chemical injury is largely responsible for most cases of limbal failure, which we also found to be the case in our study (n=15; 53.5%). Other causes included thermal burns (n=3; 10.7%) and Stevens-Johnson syndrome (n=3; 10.7%). Santos et al. evaluated 33 eyes with limbal stem cell deficiency, and of these, 67% were secondary to chemical trauma and 33% due to Stevens-Johnson syndrome. In an extensive literature review, Cauchi et al. reported that 50% of the cases of bilateral limbal stem cell deficiency were caused by chemical burns, whereas this same etiology was responsible for 100% of the cases of unilateral limbal stem cell deficiency.

The use of AM has already been well documented in the treatment of cutaneous ulcers. It was first used in ophthalmology in 1940 to treat conjunctival defects. AM works in a therapeutic manner, with specific properties that promote ocular surface repair, such as anti-adhesive and antibacterial effects, wound protection, pain reduction, and especially, in promoting reepithelialization. It can also serve as a barrier against fibroblast proliferation and exhibits low immunogenicity due to not exhibiting higher histocompatibility antigens (HLA-A, B, or DR), which prevents rejection in the host.

Some encouraging results have been published in terms of ocular surface reconstruction using AM. Its use may provide a more suitable environment for cell proliferation than the previously inflamed perilimbal environment, thus increasing the chances of successful ocular surface reconstruction. In this study, AM was used in 75% of the cases.

Another important contributing factor in corneal epithelialization in ocular surface reconstruction surgeries is tarsorrhaphy. Besides reducing tear film evaporation, it also reduces the traumatic effect caused by blinking. Tarsorrhaphy was used in conjunction with LSCT in 57% of the overall population in the study and was performed in group II in 77% of the procedures. Since group II was comprised of patients with greater clinical severity, and for the most part, with bilateral conditions, this explains the higher use of tarsorrhaphy as an adjunct in this group of patients.

The definition of surgical success in studies on limbal stem cell deficiency varies in the literature, but it mostly includes reepithelialization and visual results, which respectively indicate anatomical and functional recovery of the ocular surface. Shimazaki et al. used the detection of donor tissue-derived epithelial cells in patients who underwent heterologous LSCT combined with penetrating keratoplasty, comparing with isolated cases of penetrating keratoplasty as controls. This study suggested the long-term effectiveness of CLAL in providing epithelial cells from tissue transplanted to the ocular surface.

Ilari et al. studied 23 eyes with severe ocular surface disorders that underwent CLAL, and evaluated the reepithelialization and stabilization of the whole epithelium over a mean follow-up period of 60 months. They detected primary failure (eyes that never reepithelialized) in 24.2% of the cases. Only 21.2% of the cases retained an intact epithelium throughout the follow-up.

In another study that evaluated the results of LSCT combined with penetrating keratoplasty, Reinhard et al. detected central transparency in 30% of the grafts after 5 years of postoperative follow-up. Additionally, there was an improvement in visual acuity from 0.02 to 0.24 in patients with a clear graft and from 0.02 to 0.03 in cases of failure. The best visual acuity observed after long-term follow-up was 0.45 in patients with a clear graft and 0.26 in cases of failure.
Solomon et al. evaluated 39 eyes that underwent CLAL with AM, with or without penetrating keratoplasty, and found that the overall survival of ambulatory vision (deemed as visual acuity greater than 0.1) was 53.6% at 3 years and 44.6% at 5 years(26). A retrospective study such as the present study tends to obtain visual acuity as measured using a Snellen chart by different examiners and/or environments with non-standardized lighting. For this reason, visual acuity was not included as a criterion for evaluating surgical success. In this study, the absence of epithelial defects and conjunctivalization during postoperative follow-up were considered as variables indicating surgical success and totaled 28.5% (n=8) of the sample population. The success rate in groups I and II were 33.0% (n=5) and 23.0% (n=3), respectively. Again, it is worth noting the inclusion of patients with more severe and mostly bilateral clinical profiles in group II, requiring allogenic transplantation, which prevents the comparison of the success rates between the two groups.

The length of follow-up time may be directly related to the survival rate of limbal grafts. Santos et al. found a cumulative survival rate of 80% for CLAU and 13% for CLAL after 33 months of follow-up(9).

The survival rate of limbal grafts. Santos et al. found a cumulative survival rate of 80% for CLAU and 13% for CLAL after 33 months of follow-up(9).

Lamenting that corneal complications related to limbal transplantation account for most failures of the procedure, Santos et al. (2015) compared the success of autologous and allogeneic limbal transplantation in a prospective study. The results showed that the survival rate of autologous limbal grafts was significantly higher than that of allogeneic limbal grafts (95.6% vs. 68.7%, P<0.01). The authors suggested that the use of autologous limbal grafts is preferred in cases of limbal stem cell deficiency to achieve better outcomes.

REFERENCES