

Excision, cryotherapy and mitomycin c for the treatment of conjunctival-corneal intraepithelial neoplasia

Excisão, crioterapia e mitomicina C no tratamento da neoplasia intraepitelial córneo-conjuntival

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ABSTRACT

Purpose: To evaluate the safety and efficacy of three different techniques for the treatment of conjunctival-corneal intraepithelial neoplasia. **Methods:** Twenty-six eyes of 26 patients, 11 women and 15 men were included in a nonrandomized, retrospective, observational case series. Mean patient age was 64 years (range, 32-88 years). All patients were treated from 1999 to 2014. Mean postoperative follow-up was 4 years (range, 3 months to 14 years). All patients underwent excision of the lesion with a 4-mm safety margin and a 2-mm from the limbus cryotherapy. As further treatment the patients were divided into three groups: Group 1 (8 eyes): conjunctival autograft and 2 cycles of mitomycin C 0.02% (MMC) eye drops, four times daily for 10 days with an interval of 30 days between cycles. Group 2 (9 eyes): amniotic membrane (AM) graft and 3 cycles of MMC eye drops, three times daily for 10 days with an interval of 10 days between the 1st and 2nd cycle and 30 days between the 2nd and 3rd cycle. Group 3 (9 eyes): AM graft without MMC eye drops. **Results:** After a single treatment, the lesions were eradicated in twenty-three patients. Recurrence was noted in three patients from Group 3 at 3 months, 6 months and 1 year after treatment. Two of them were retreated by the technique used in Group 1 without further recurrence; the other patient underwent enucleation. No adverse effects related to the use of mitomycin C were observed during. There were no side effects related to the use of mitomycin C during follow-up. **Conclusion:** After surgical excision and cryotherapy, patients treated with conjunctival or amniotic membrane graft associated with mitomycin C, showed a tendency to more effective results.

Keywords: Eye neoplasms/drug therapy; Conjunctival neoplasms/drug therapy; Mitomycin /therapeutic use; Amnion

RESUMO

Objetivo: Avaliar a segurança e eficácia de três técnicas distintas para o tratamento de neoplasia intraepitelial córneo-conjuntival. **Métodos:** Vinte e seis pacientes, 11 mulheres e 15 homens, com idade entre 32 e 88 anos (média 64,84 anos), atendidos entre 1999 e 2014, foram incluídos neste estudo. Todos os pacientes foram submetidos à exérese da lesão com margem de segurança de 4mm e crioterapia a 2mm do limbo. Conforme o tratamento complementar, os pacientes foram divididos em três grupos: Grupo 1 (8 olhos): recobrimento conjuntival e 2 ciclos de mitomicina C 0,02% (MMC) 4x/dia por 10 dias, com intervalo de 30 dias entre os ciclos. Grupo 2 (9 olhos): recobrimento com membrana amniótica e 3 ciclos de MMC 3x/dia por 10 dias, com intervalo de 10 dias entre o 1º e o 2º ciclo e 30 dias entre 2º e o 3º ciclo. Grupo 3 (9 olhos): recobrimento com membrana amniótica sem o uso de MMC. O acompanhamento dos pacientes variou de 3 meses a 14 anos (média: 4 anos). **Resultados:** Vinte e três pacientes apresentaram erradicação da lesão com um único tratamento. Três pacientes do Grupo 3 apresentaram recidiva da lesão após 3 meses, 6 meses e 1 ano do tratamento. Dois deles foram retratados pela técnica 1, sem nova recidiva após seguimento de 3 e 4 anos; o outro paciente foi submetido à enucleação. Não foram observados efeitos colaterais relacionados ao uso da mitomicina C durante o seguimento. **Conclusão:** Após exérese da lesão e crioterapia, os pacientes tratados com recobrimento conjuntival ou membrana amniótica associado à mitomicina C mostraram tendência a resultados mais eficazes.

Descritores: Neoplasias oculares/quimioterapia; Neoplasias da conjuntival/quimioterapia; Mitomicina/uso terapêutico; Amnion

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INTRODUCTION

The intraepithelial neoplasia and squamous cell carcinoma of the cornea and conjunctiva are the most common tumors of the ocular surface⁽¹⁾. It is a benign disease of the epithelium of the ocular surface, with low potential for malignancy, difficult to diagnose (presented in the most varied forms) and difficult to treat⁽²⁾. It is characterized by variation in degrees of dysplasia (I to III) according to the extent of involvement of the epithelium and carcinoma *in situ*, in which atypical cells reach the entire thickness of the epithelium^(3,4).

Its incidence is approximately 1.9 cases per 100,000 people per year, and represents about 1/3 of all epithelial lesions of the conjunctiva acquired under the surgery^(1,5).

The form of presentation ranges from well-defined lesions, with gelatinous or leukoplakiaspect to more diffuse and pagetoid forms⁽⁶⁾. It is presented as a unilateral, slowly progressive lesion with low malignant potential⁽³⁾.

The classification of these neoplasms is made according to its location in relation to the epithelial basement membrane. Thus, these tumors are divided into conjunctival and/or corneal (NIC) intraepithelial neoplasia when they are confined to the epithelium, and squamous cell carcinoma (SCC) when there is invasion of the epithelial basement membrane and the substance itself⁽⁶⁾.

The clinical distinction between NIC and SCC is hard⁽⁷⁾. The clinical manifestations of NIC and SCC may be foreign body sensation, eye irritation, conjunctival hyperemia, and growth of gelatinous or leucodysplastic papillary lesions, usually accompanied by nourisher vases, preferably in the limbic region. The NIC has slow development and low malignant potential, and the exact frequency of progression to SCC is not known⁽⁸⁾.

Invasive squamous cell carcinoma (SCC) is the most common malignancy of the conjunctiva. Neoplastic cells disrupt the basement membrane of the epithelium of the conjunctiva, invading the lamina propria capable of causing metastasis^(4,5).

The etiology of NIC and SCC seems to be multifactorial⁽⁹⁾. Among the risk factors proposed are advanced age⁽⁶⁾, exposure to ultraviolet rays^(6,10,11), oil products (gasoline and diesel), cigarette smoke and infection by the human papillomavirus (HPV)⁽⁶⁾ and human immunodeficiency virus (HIV)^(12,13).

The treatment of choice for NIC and the SCC consists of excisional biopsy associated to cryotherapy, in an attempt to reduce the tumor recurrences^(14,15), being incomplete resection the biggest risk factor for therapeutic failure.^(6,7-16)

Adjuvant treatments such as cryotherapy and radiation have been used in an attempt to reduce post-surgical resection relapse⁽⁸⁾. Another method recently employed as primary or adjunctive therapy is the use of chemotherapeutic agents, such as interferons, mitomycin C and 5-fluoracil, being useful in diffuse disease, in addition to avoiding the complications associated to surgical intervention such as corneal-conjunctival scars, corneal-scleral melting, and formation of retrocorneal membrane⁽⁸⁾. The side effects observed are: mild hyperemia, ocular allergy, tearing, photophobia, punctate epitheliopathy, and pain. Slow growth, high rates of recurrence, and potential for malignancy ensure that NIC and SCC patients treated must be followed up indefinitely. In advanced cases, enucleation or exenteration may be necessary⁽¹⁷⁾.

The aim of this study is to report the results obtained after excision of the lesion and cryotherapy in three distinct

approaches: conjunctival covering and 1 cycle of mitomycin C, covering with amniotic membrane and 3 cycles of mitomycin C, or amniotic membrane without the use of mitomycin C.

METHODS

The present study included 26 eyes of 26 patients, being 11 females and 15 males with cornea and conjunctiva lesions, divided into three groups: Group 1 (8 eyes), Group 2 (9 eyes), and Group 3 (9 eyes), and treated with three different techniques.

All the lesions were subject to histopathological examination to confirm the diagnosis of corneal-conjunctival intraepithelial neoplasia, with free surgical margins.

Patients in Group 1 underwent excision of the lesion with a safety margin of 4 mm, cryotherapy at 2 mm of limbo, conjunctival covering, and they were treated with 2 cycles of eyedrops Mitomycin C 0.02% 4 times a day for 10 days, with a 30-day interval between the cycles.

Patients in Group 2 underwent excision of the lesion with a safety margin of 4 mm, cryotherapy at 2 mm of limbo, conjunctival covering, and they were treated with 3 cycles of eyedrops Mitomycin C 0.02% 3 times a day for 10 days, with a 10-day interval between the first and second cycles, and a 30-day interval between the second and third cycles.

Patients in Group 3 underwent excision of the lesion with a safety margin of 4 mm, cryotherapy at 2 mm of limbo, and covering with amniotic membrane, without the use of Mitomycin C.

Post-operative visits were made every 3 months during the first 12 months after the end of treatment, and every 6 months after this period. Topic antibiotics and steroids were prescribed postoperatively, in addition to artificial tears.

This study was carried out at Instituto de Olhos de Goiânia, and approved by the local Ethics Committee. All participants signed the informed consent form about the study.

RESULTS

We treated 26 eyes of 26 patients with intraepithelial neoplasia.

Twenty-three patients had eradication of the lesion with a single treatment. Three patients in Group 3 presented recurrence of the lesion after 3 months, 6 months and 1 year of treatment. Two of them were portrayed by technique 1 without further recurrence after follow-up of 3 and 4 years. Another patient underwent enucleation due to recurrences with progression to squamous cell carcinoma, with invasion of the limbic sclera and cameralar sinus structures.

This study was carried out in prospective way. The average age was 64.84 years, varying between 32 and 88 years. The size of the lesions ranged from 12 - 48 mm². Among the 26 eyes, 12 (46.2%) were from white patients, 8 (30.8%) from brown patients, and only 6 (23.1%) from black patients (Table 1). The patients underwent three different treatment techniques previously explained, and each with a different time of progression, with an average of 67% of patients without relapse and 83% of patients with relapse. Due to the small number of patients in each group, the statistical analysis by the Wicoxon test failed to identify statistically significant difference between the groups. However, there was a tendency to lower recurrence rate in groups 1 and 2.

Table 1
Patients data

Variable	Without recurrence	With recurrence	p-Value
Age (years)	64 (55-72) (N=23)	69 (68-73) (N=3)	0.301
Lesion-mm ²	21 (12-31) (N=20)	46.5 (45-48) (N=2)	0.130
Progression time n(%)	67% (N=23)	83% (N=3)	0.688
Gender (n)			0.556
Female	9	2	
Male	14	1	
Color (n)			0.294
White	9	3	
Black	6	0	
Brown	8	0	

Table 2
Follow-up time

	Technique = 1 (N=8)	Technique = 2 (N=9)	Technique= 3 (N=9)	p-Value
Time (years)	4.5 (3.5-5.5)	3 (3-5)	2 (1-5)	0.356

The techniques had different follow-up times and different relapse rates. Group 1 had a follow-up of about 4.5 years (3.5 - 5.5), Group 2 of 3 years (3 - 5), and Group 3 of 2 years (1 - 5), each with a “n” of 8, 9 and 9 respectively (Table 2). The significance level of 5% did not show significant difference in the medians of the follow-up time among the 3 techniques (exact Kruskal-Wallis Test, p= 0.356).

Table 3 shows that among the three techniques Group 3 was the only one presenting relapses, with a total of 3 (33.3%) of the total sample of this group. In other groups, we did not observe relapses, being impossible to prove significance by the Fisher-Freeman-Halton test, with a p of 0.086, due to the small number of eyes, following just a tendency to relapse when using only the excision with a 4-mm safety margin associated with cryotherapy at 2 mm of limbo associated to coating of the amniotic membrane without mitomycin c.

Table 3
Relapse

Variable	Technique =1 (N=8)	Technique =2 (N=9)	Technique=3 (N=9)	Total	p-Value
Relapse					0
8 (100.0%)	9 (100.0%)	6 (66.7%)	23 (88.5%)	0.086	1
0 (0%)	0 (0%)	3 (33.3%)	3 (11.5%)		

Quantitative variables were compared between Groups 2 and 3 independent, according to the exact test of the sum of Wilcoxon and exact test Kruskal-Wallis respectively.

Quantitative variables and categorical variables were described with median (interquartile range) and counts (percentages) respectively. The software R (R Foundation, Vienna, Austria) was used in the statistical analysis of data. All significance

possibilities presented are of bilateral type, and values less than 0.05 were considered statistically significant. P-values between 0.05 and 0.10 were interpreted as marginally significant.

DISCUSSION

The present study examined various techniques for the treatment of corneal-conjunctival intraepithelial neoplasia (NIC), since, although a benign lesion, it is also premalignant with different recurrence rates depending on the technique used for the treatment and the social group analyzed. The NICs are classified into: NIC I (mild dysplasia), NIC II (moderate dysplasia), NIC III (severe dysplasia or carcinoma *in situ*) according to the extent of the lesion, degree of atypia and loss of cell polarity. When there is basement membrane invasion, the lesion is called invasive squamous cell carcinoma (SCC)^(1,8).

The SCC represents 44.7% to 61.8%⁽⁶⁾ of malignant lesions of conjunctiva, and is considered the most common malignancy of the conjunctiva. It is a low degree of malignancy, rarely leading to metastasis, and responds very well to surgical excision. However, recurrence may occur, which makes it more aggressive⁽³⁾.

Clinically, it is difficult to distinguish between NIC and SCC. Nevertheless, the SCC is considered a more advanced lesion than *in situ* carcinoma, also being called “invasive” carcinoma, as it surpasses the basal membrane. Nevertheless, *in situ* carcinoma may develop into SCC and may also recur, as observed in this study in one of the patients in group 3, which leads us to consider it as an important lesion as CEC^(8,9,17).

It is predominant in males, caucasians, residents in latitude below 30° degrees in latitude from Ecuador and elderly, especially after the fifth decade of life.^(18,19)

Our work showed higher prevalence of NIC in male patients, among the 26 eyes of this pathology. The age of patients ranged from 55 to 73 years, and there was recurrence in patients with an average age of 69 years.

As for the lesions, they have different shapes, though generally they are slightly elevated, unilateral, well demarcated, and with normal adjacent tissue. Usually they are accompanied by important vascularization surrounding the lesion, and the aspect may be gelatinous or covered by keratin. Its most frequent location is the bulbar conjunctiva, in the nasal limbo, and less often occurs in the interpalpebral fissure. In the form of leukoplakia, the lesion presents the form of a white board on the surface of the lesion due to secondary hyperkeratosis, and sometimes with papillomatous aspect.

Although the ocular surface squamous neoplasia (OSSN) has a benign course, with low rate of metastasis, there is a high rate of recurrence of these lesions.

Several techniques for the treatment have been reported, but in 1/3 of the patients there is a need for repeated surgeries due to recurrence of the lesion or the presence of residual lesion.

Traditionally, surgical excision associated to cryotherapy has been the treatment. Cryotherapy can cause cell death due to fast freezing followed by a slow thaw, and is applied to the margin of limbo and on the edges of the conjunctiva after excision of the lesion. It may have complications such as corneal superficial scarring, iritis, endothelial dysfunction and edema, and should be reserved only for the peri-limbal lesions.⁽²⁰⁾ In this study, no

complications secondary to cryotherapy were observed.

The presence of compromised surgical margins to the histopathological exam is frequent. Therefore, the lesion should always be resected with good safety margin, as the most important risk factor for recurrence is the presence of compromised surgical margins.⁽²¹⁾ It is very important to prevent direct manipulation of the tumor during surgery to prevent seeding of tumor cells to a new area. The recurrence rate is variable and depends on the surgical technique, conditions for surgical margins and the use of adjuvant therapies. In this series of operated patients, all lesions were subjected to histopathological examination which confirmed free surgical margins.

The topical use of mitomycin C (MMC) has been shown to be effective in the treatment of NIC and SCC, with few side effects and low rates of recurrence^(20,22). It is a potent, toxic antimycotic, which inhibits DNA synthesis, and can be used as adjunctive therapy to surgical excision or separate use in primary and recurrent disease. It can present side effects like mild discomfort, ocular hyperaemia, photophobia, epithelial punctate keratitis, and lacrimation⁽²⁰⁾. When used at a dose of 0.02%, MMC eradicates most injuries and has low toxicity if used for up to 14 days. After that, it causes pain, blepharospasm, conjunctival hyperemia, symblepharon, corneal erosions and uveitis. This way, we recommend cycles of up to 14 days of topical administration of the drug, with an interval of at least ten days, and a maximum of three cycles may be prescribed. Our study did not observe present complications related to the use of Mitomycin C 0.02%.

In vitro experiments showed that the stromal matrix of amniotic membrane suppresses the activity of the growth factor beta (TGF- β) and myofibroblastic differentiation of corneal fibroblasts, human limbo and conjunctiva⁽²³⁾.

Amniotic membrane transplantation offers an advantage over other types of mucosal graft, as it does not induce scarring in the donor and creates in the receiver a surface for proliferation of cells with phenotype of normal conjunctival epithelium, demonstrated by studies employing impression cytology. Another advantage of the use of amniotic membrane graft rather than labial mucosa is the transparency that the former presents, which allows examination of deeper structures postoperatively, and any relapse that may occur in the graft may be detected earlier.^(24,25)

The amniotic membrane is constituted by a thick layer of collagen, laminin basement membrane and the type IV collagen⁽²⁶⁾. This composition promotes healing in persistent epithelial defects to facilitate the adhesion and migration of basal epithelial cells. Also, it shows excellent angiogenic, antimicrobial, anti-inflammatory action. The authors mentioned before described that the amniotic membrane does not induce immune rejection, for not expressing histocompatibility antigens (HLA-A, B or DR)⁽²⁷⁾.

The rate of relapse in 5 years was described in different ways according to the work carried out previously, being identified recurrences in 26%⁽²⁸⁾, 52%⁽²⁹⁾ and 60, 7%⁽³⁰⁾, and this difference can be explained by the percentage of tumors treated with adjuvant therapy in the different studies.

In our study, we found no statistically significant difference between the groups due to the small number of patients in each

group. However, recurrence was observed only in Group 3, which underwent coating with amniotic membrane without the use of mitomycin C. While in most cases the tumor invades the surface more than the depth of the tissue of origin, the scleral, intraocular, orbital invasion, and even spread is not uncommon. The scleral involvement may occur in 37% of cases, intraocular in 2% to 8%, and orbital invasion in 12% to 16%⁽⁴⁾. The enucleation would be indicated for cases of intraocular invasion and exenteration for patients with orbital involvement. In the present study, there was no need of enucleation in one of the patients, due to progression to squamous cell carcinoma with invasion of sclera and structures of ciliary sinus. Studies observed until 4% of metastasis to the parotid gland and cervical lymph nodes, and distant metastasis seems to be even less common.

The relapse can arise many years after resection of the tumor, and may vary from one month up to eleven years. The follow-up of patients with NIC must be careful, and may be done at least one annual monitoring for life, as injuries are frequent in our world, being potentially serious with the possibility of relapse. This study seeks to stimulate the development of new studies that investigate the ideal time and the ideal dosage for use of MMC in cases of NIC, as well as its therapeutic efficacy and long-term complications.

CONCLUSION

The results of this study showed that the topical use of MMC at 0.02% associated to conjunctival or amniotic membrane coatings showed, in a first moment, safe and effective for the treatment of NIC in the therapeutic modalities presented, demonstrating different approaches to treat such eye disease.

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