Algorithms for eyelid repair

Algoritmos para reparo das pálpebras

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ABSTRACT

Eyelid defects resulting from skin cancer excision are common in the daily practice of dermatologic surgeons.

The aim of this review is to summarize the most relevant methods for eyelid repair, proposing reconstructive algorithms for both lower and upper eyelids. Both algorithms were designed according with dichotomic decisions based on the thickness (partial- versus full--thickness) and the size of the eyelid defect (less than 1/3, less than 2/3 or larger than 2/3 of the eyelid length).

Keywords: Eyelids; Reconstruction; Eyelid Neoplasms; Eyelid Neoplasms/surgery; Cornea

RESUMO

Os defeitos nas pálpebras resultantes da excisão do câncer de pele são comuns na prática diária dos cirurgiões dermatológicos.

O objetivo desta revisão é resumir os métodos mais relevantes para o reparo da pálpebra, propondo algoritmos reconstrutivos para as pálpebras inferiores e superiores. Ambos os algoritmos foram projetados de acordo com decisões dicotômicas baseadas na espessura (espessura parcial versus total) e no tamanho do defeito da pálpebra (menor que 1/3, menor que 2/3 ou maior que 2/3 do comprimento da pálpebra).

Palavras-Chave: *Pálpebras; Reconstrução; Cornea; Neoplasias Palpebrais; Neoplasias Palpebrais/cirurgia*

Review Articles

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INTRODUCTION

Skin cancer excision is the main cause of eyelid defects to be treated by dermatologic surgeons. As occurs in other facial regions, basal cell carcinoma (BCC) is the most frequent neoplasm in the eyelids. Squamous cell carcinoma (SCC), melanoma and sebaceous carcinoma account for most of the remaining cases.[1] The dermatologic surgeon has to be prepared to face this kind of defects, for which is essential to develop advanced surgical skills based on solid knowledge about eyelid anatomy and physiology.

Eyelid reconstruction is usually challenging and the decision about the optimal method to repair a specific defect is mainly based on two factors: the thickness and the extent of the defect.

The chosen closure method should result in tension vectors with a predominant horizontal orientation. Furthermore, the alignment of the free margin and the canthal fixation should be preserved or restored. Each layer of the eyelid should be repaired, from the internal tarsoconjunctival layer to the external cutaneous layer. These basic principles are essential to achieve optimal cosmetic and functional results, preventing complications, such are ectropium, lagophthalmos, epiphora, chronic conjunctivitis and corneal dryness, and ulceration.

Reconstructive algorithms are generally considered useful tools in the clinical setting, helping the surgeon to decide which management strategy should be chosen. The aim of this work is to design decisional algorithms both for upper and lower eyelids repair.

MATERIAL AND METHODS

The algorithms were based on a literature review about eyelid repair and also on the author personal experience. Therefore, despite the literature review supporting the proposed options, the algorithms' decisions were also influenced by some personal preferences. The algorithms have no intention to include an extensive review of all the possible reconstructive options reported in the literature. Under the same combination of criteria (personal experience and literature review), the most relevant techniques were considered as the most reliable and consistent.

RESULTS

Conditionals of the algorithms

The size and the thickness of the defect were identified as the two factors with more impact in the decision about the reconstructive method to be used for both lower and upper eyelid defects.[2,3,4]

The management of partial-thickness defects differs significantly from the management of full-thickness defects since the latter require restoration of the posterior lamella (tarsoconjunctival layer) in addition to the anterior lamella (myocutaneous layer)[2,3,4]. Therefore, the thickness was considered the first condition to be included in the algorithm.

The extent of the eyelid length affected by the defect is more relevant than the absolute diameter itself. Therefore, the size, expressed as a fraction of the eyelid length, was pointed out as the second condition of the algorithm.

The ability to close primarily a defect is mainly related to the size of the defect. However, it may be also influenced by individual factors (tissue elasticity, age, etc.). Therefore, in those situations in which individual variations were considered to have influence in the reconstructive strategy, the ability to primarily close a defect was included as a specific condition instead of the size itself.

Partial-thickness defects. The repair of skin-only defects on the eyelid should be performed avoiding vertical tension to prevent ectropion, scleral exposure or lagophthalmos, causing cosmetic impairment and functional abnormalities such as epiphora, chronic conjunctivitis, and dry eye. Upper eyelids admit some grade of vertical tension, but lower eyelids are unable to support any kind of vertical tension. Therefore, a horizontal approach should be always considered.[5] If the primary closure leads to distortion or vertical tension over the eyelid margin, the need for special techniques is obvious. Patients with impaired snap-back test are more prone to the occurrence of ectropium.[6]

In large defects occurring in the lower eyelid, direct closure can be sometimes possible after stabilization of the eyelid through a lateral canthopexy and a lifting of the suborbicular oculi fascial tissue (SOOF) (figure 1), anchoring this tissue to the periosteum of the orbital rim.[7] Otherwise, a flap or a graft will be needed. Full-thickness skin grafts are efficient for repairing the anterior lamella on both eyelids.[3,4] The most used donor area is the opposite upper eyelid.

The redundant skin of the upper eyelid can be recruited as advancement or rotation flaps for upper eyelid partial-thickness defects. Transposition flaps from the periocular region or from the supraciliary region can also be harvested for repairing bigger defects.

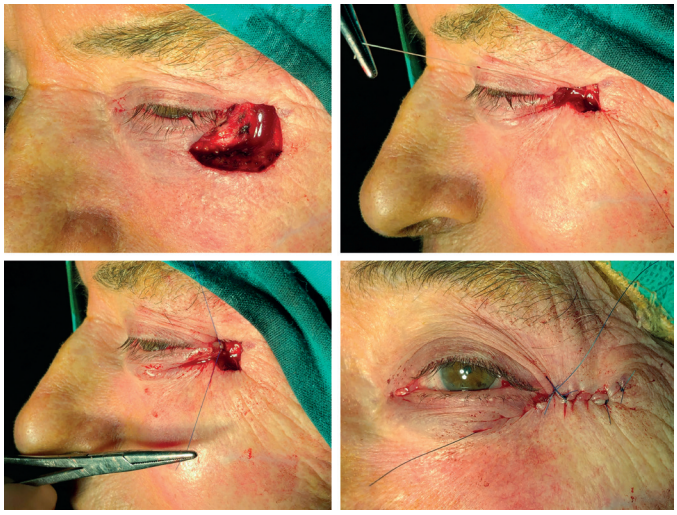

Figure 1: Direct closure of a large partial thickness defect of the lower eyelid after lateral canthopexy and elevation of suborbicular ocular fat

Several flaps were described for repairing lower eyelid partial-thickness defects. The Tripier flap[8], which consists of a myocutaneous transposition flap from the homolateral upper eyelid is one of the most reliable techniques for this purpose (Figure 2). Other alternative transposition flaps are Fricke flap, Kreibig flap, and superiorly-based nasolabial flap. Advancement flaps (McGregor flap and Imre flap)[8,9] from the periocular region are also a possibility. Mustardé rotation flap[10] is a very good option to repair extensive defects of the lower eyelid anterior lamella (Figure 3).

Full-thickness defects. Small full-thickness defects can be repaired by direct closure. If the defect involves the eyelid margin, usually the closure is performed after planning the excision under a pentagonal shape (Figure 4, left column). This method results in a hipereversion of the lesion borders, leading to a correct alignment of the eyelid margin, crucial to achieve an optimal cosmetic and functional outcome.[11] If there is too much tension and it is hard to join the lesion borders, the closure can be easily performed after the lateral canthal ligament have been cut (lateral cantholysis).[4] This will allow an additional advancement of up to 5 mm (Figure 4, right column). After the suture, the lateral canthal ligament should be reattached to the periosteum of the orbital rim.

The Tenzel flap (semicircular advancement-rotation flap from the zygomatic region)[4] is an excellent option for defects up to 1/3 of the eyelid length, despite this flap may be sufficient to repair defects up to 40-50% of the eyelid length in some older patients. The Tenzel flap has the advantage to repair both lamellas within a single procedure. The semicircular design of this flap is highly important to prevent ectropion. The convexity of the semicircle is superior for the lower eyelid; however, it is considered inferior for the upper eyelid (Figure 5).

Defects bigger than 1/3 of the lower eyelid length commonly require multi-step procedures with sequential repair of posterior and anterior lamellas. Tarsoconjunctival grafts harvested in the upper eyelid (Figure 6, upper line) or flaps (Hughes flap)[12] can repair defects up to 2/3 of the lower eyelid extent (Figure 7). From 2/3 to the totality of the eyelid, the nasal septum (alternatively, the oral mucosa and auricular cartilage can be used) is a good donor site to harvest chondromucosal grafts (Figure 6, bottom line), big enough for the entire posterior lamella restoration.[13] When the posterior lamella is repaired with a graft, a flap should be performed to repair the anterior lamella,[4] since a graft sutured over a graft will result in poor nutrition of both grafts, leading to necrosis. However, a full-thickness skin graft is

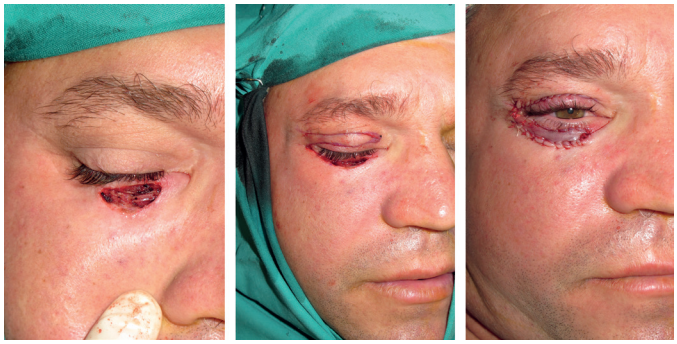

Figure 2: Myocutaneous transposition flap (Tripier) transposed from the upper eyelid for lower eyelid repair

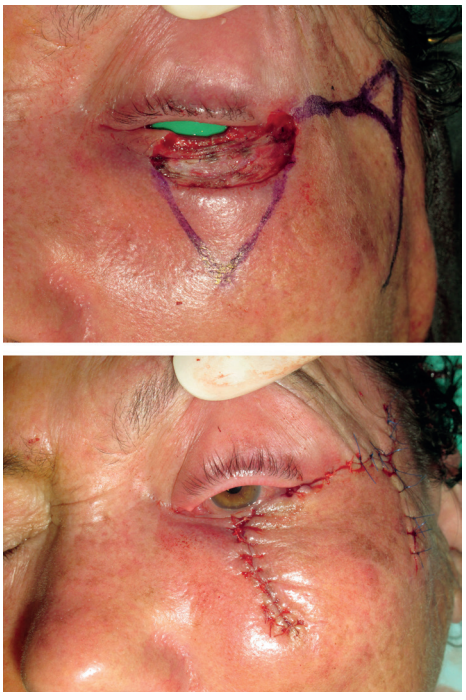

Figure 3: Mustardé rotation flap for lower eyelid repair

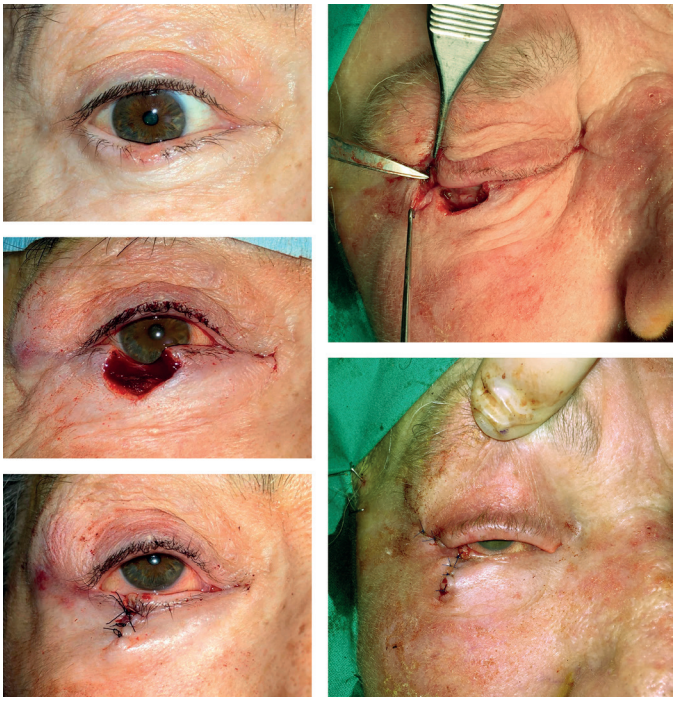

Figure 4: Pentagonal excision of the lower and upper eyelids (left column). Lateral canolysis, allowing an additional movement of the eyelid that allows direct closure (right column)

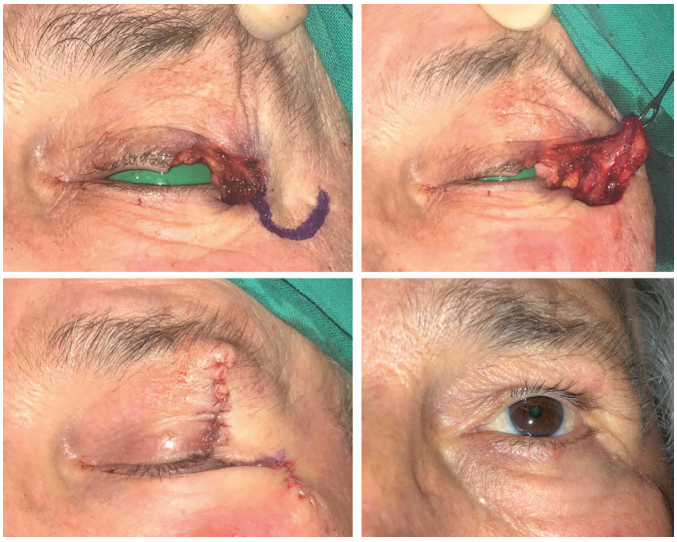

Figure 5: Inverted Tenzel flap for upper eyelid repair

a safe procedure to perform in addition to a Hughes tarsoconjuctival flap.

Defects larger than one half of the upper eyelid length are very difficult to repair and the options are very limited. The most reliable and safe procedure is the Cuttler-Beard flap,[14] which consists of an advancement full-thickness flap harvested in the lower eyelid. Like the Hughes flap, it is an interpolated flap with a 4-6 weeks period before pedicle division. Since the Cuttler-Beard flap should not affect the lower eyelid margin, it is tunneled to achieve its final position over the upper eyelid defect.

Algorithms. The final algorithms can be analyzed in the Figures 9 and 10.

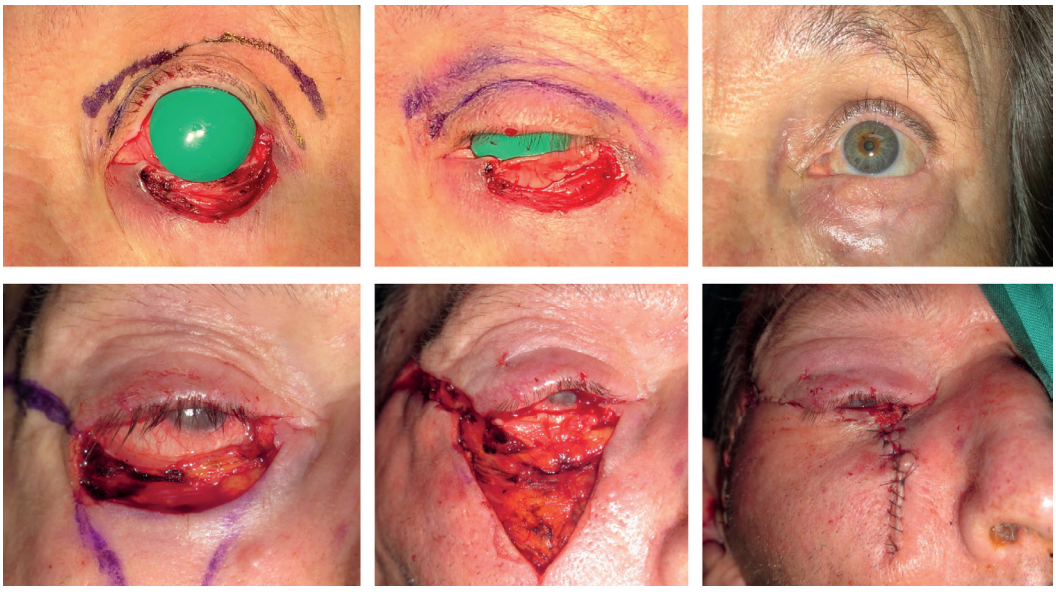

Figure 6:
Posterior lamella repair. Tarsoconjunctival graft (top) combined with a Tripier flap for anterior lamella. Chondromucosal graf (bottom) combined with a Mustardé flap

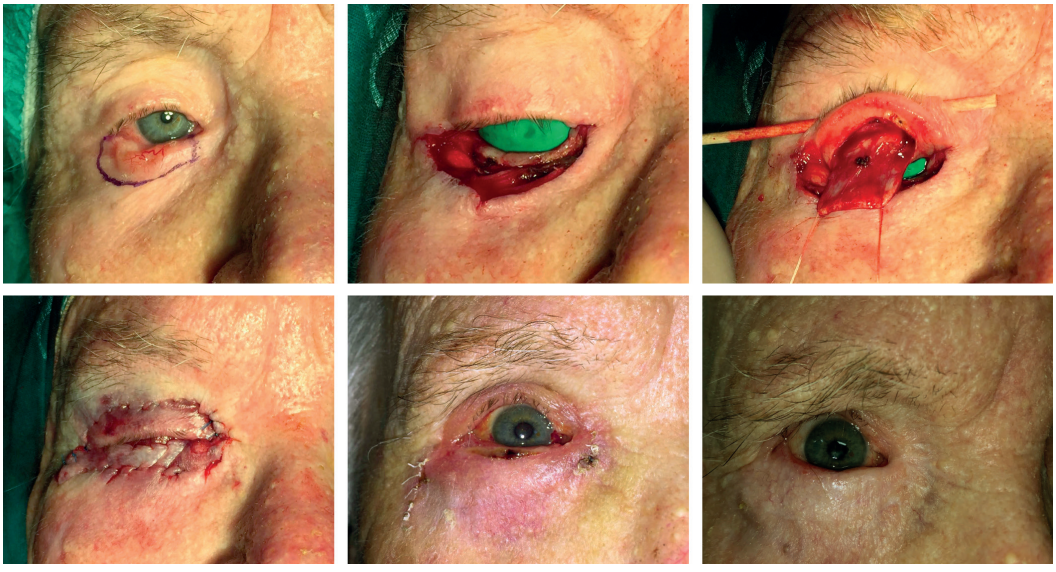

Figure 7:
Hughes flap combined with a Tripier flap for repair a full-thickness defect of lower eyelid

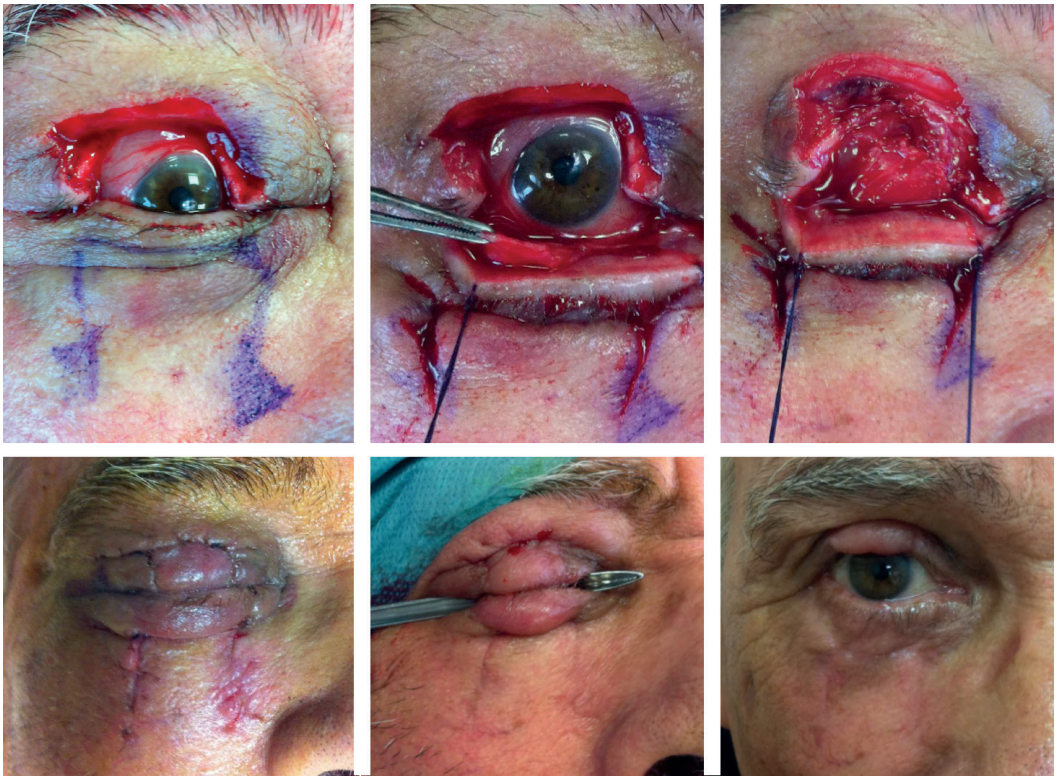

Figure 8:
Cuttler-Beard flap for a large upper eyelid full-thickness defect

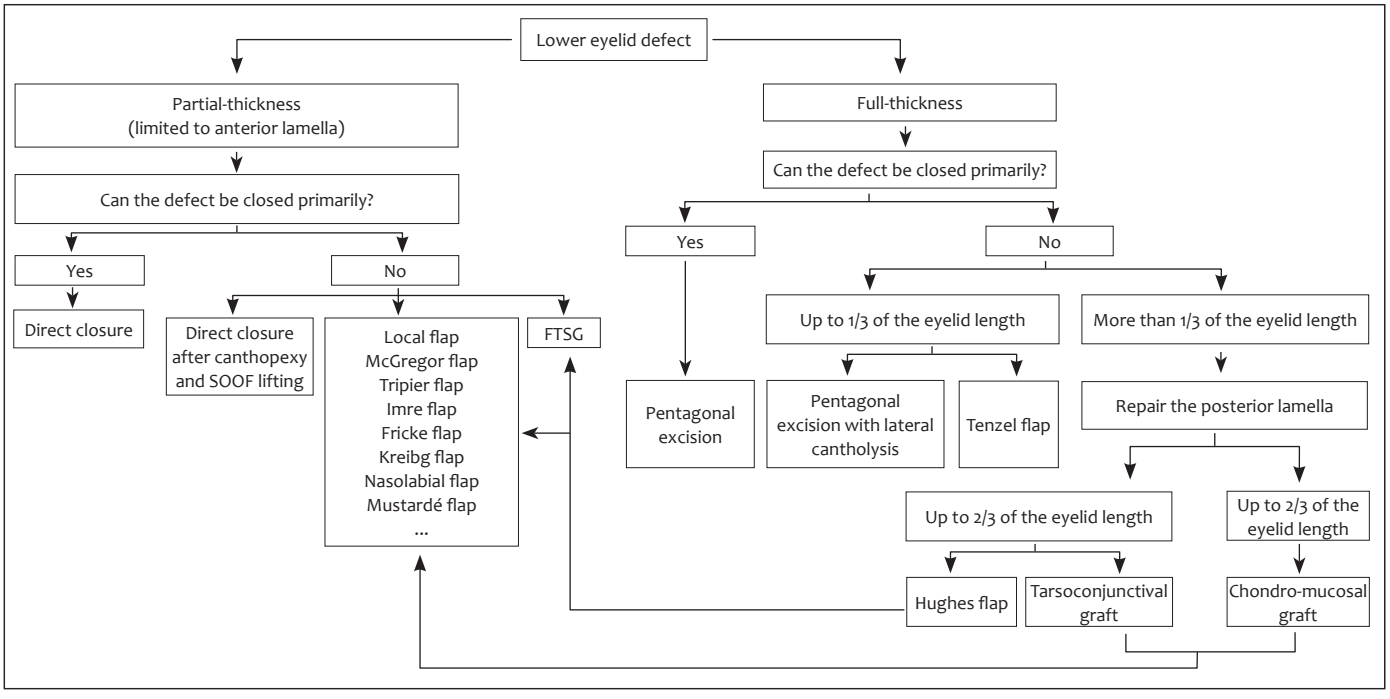

Figure 9: Reconstructive algorithm for lower eyelids

CONCLUSIONS

Eyelid reconstruction is challenging due to the anatomic and physiologic particularities of the eyelids. The multiplicity of surgical techniques available, differences in defects thickness and size, and individual variations in skin mobility and elasticity make the reconstructive strategy difficult to establish.

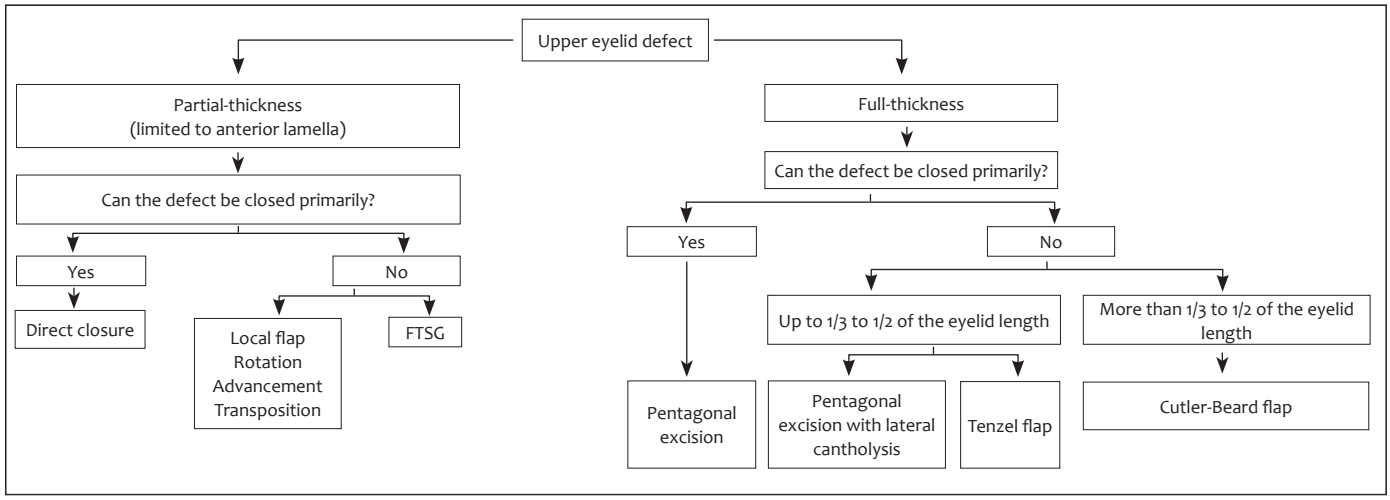

FIGURE 10: Reconstructive algorithm for upper eyelids

The proposed algorithms summarize the most common reconstructive procedures for upper and lower eyelids defects, sequentially categorized under a dichotomic decision tree aiming to assist the dermatologic surgeon during the reconstructive decision-making process.

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Updates on the cosmiatric and therapeutic use of botulinum toxin

Atualizações do uso cosmiátrico e terapêutico da toxina botulínica

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ABSTRACT

Botulinum toxin has transient flaccid neuromuscular paralysis as its mechanism of action. Recent studies are identifying new ways to use botulinum toxin for a variety of purposes, both in the aesthetic and in the therapeutic field. This work aimed to conduct a literature review on these applications. In the aesthetic field, botulinum toxin has shown benefit in the treatment of hypertrophic scars, rejuvenation of the scrotal region, definition of the gastrocnemius muscle, and microdoses use. In the treatment of pathologies, the review has shown that botulinum toxin may be useful for the treatment of post-herpetic neuralgia and other pain syndromes, craniofacial hyperhidrosis, rosacea, and Hailey-Hailey disease.

Keywords: Botulinum toxins; Botulinum toxins, Type A; Esthetics; Pruritus; Rejuvenation; Cicatrix; Cicatrix, Hypertrophic

RESUMO

A toxina botulínica tem como mecanismo de ação a paralisia neuromuscular flácida transitória. Estudos recentes estão identificando novas formas de uso da toxina botulínica para diversos fins, tanto no campo estético quanto no terapêutico. Este trabalho teve como objetivo realizar uma revisão bibliográfica sobre essas aplicações. No âmbito estético, a toxina botulínica demonstrou benefício em tratamento de cicatrizes hipertróficas, rejuvenescimento da região escrotal, definição do músculo gastrocnêmio e sendo usada em microdoses. Já no tratamento de patologias, a revisão demonstrou que a toxina botulínica pode ser útil para tratamento da neuralgia pós-herpética e de outras síndromes álgicas, da hiperidrose craniofacial, da rosácea e da doença de Hailey-Hailey.

Palavras-Chave: *Toxinas botulínicas tipo A; Estética; Toxinas botulínicas; Prurido; Rejuvenescimento; Cicatriz; Cicatriz hipertrófica*

Review Articles

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INTRODUCTION

Botulinum toxin (BT) is a neurotoxin produced by the anaerobic bacterium *Clostridium botulinum*, which causes botulism, a serious disease characterized by the paralysis of the facial muscles, limbs and even respiratory muscles, which can lead to death. The mechanism of action of botulinum toxin is to determine transient flaccid neuromuscular paralysis through the chemical denervation process. Thus, it binds to the presynaptic receptor in the neuromuscular junction; the complex formed is endocytosed, followed by synaptosomal-associated protein-25 cleavage (SNAP-25), which culminates in the blockade of acetylcholine release, preventing muscle contraction until the function is repaired again in approximately three to six months.[1,2,3]

Botulinum toxin can be differentiated into eight serotypes named A, B, Cb, C2, D, E, F, and G. The types A and B are commercially available. In dermatology, botulinum toxin type A is the most used since the FDA approved it in 2002.[3]

Botulinum toxin was used therapeutically for the first time in the 1960s by ophthalmologists in San Francisco to correct strabismus.[2] Later, new studies were conducted and its use was progressively expanded to other therapeutic areas until, finally, in 1989, it was used for the first time with an aesthetic purpose, initially to correct asymmetries by facial paralysis and later, in 1992, for correction of expression wrinkles.[2]

In esthetics, it has a well-defined use to treat glabellar and periorbital wrinkles. However, more recent research with new application techniques, formulations, and use in combination with fillers and other procedures has revealed its potential for better aesthetic results, new uses, and increased patient satisfaction.[1]

With non-aesthetic goals, botulinum toxin has been used to treat inflammatory skin diseases such as acne, rosacea, psoriasis, diseases caused or exacerbated by hyperhidrosis, and to improve the appearance of post-surgical scars.[1]

The objective of this review is to report the new uses of botulinum toxin both in aesthetics and in the therapeutic field.

Aesthetic uses

Botulinum toxin is an established option to minimize signs of aging and tired facial appearance. Its use is mainly related to the musculature of the facial mime. However, new cosmetic applications have shown excellent results and should be better known and incorporated into the dermatologist practice.[3,4]

Wound healing

Wound healing is a complex and dynamic process dependent on the coordinated activity of multiple cells. It consists of three overlapping phases: the initial inflammatory (or migration) phase, lasting a few days, during which cytokines and growth factors recruit inflammatory cells; the proliferative (or mitotic) phase, lasting few weeks and characterized by the formation of granulation tissue, composed of fibroblasts that synthesize the extracellular matrix and by myofibroblasts that initiate contraction; and the final stage of maturation, lasting about seven months, which begins when the wound is closed. At this stage, the scar begins to retract and the edema decreases, the inflammatory cells gradually reduce in number, the extracellular matrix is degraded, the angiogenesis ceases, and the type III (immature) collagen is modified in type I (mature) collagen.[1,3]

Wound healing is an imperfect process, which can lead to elevated (hypertrophic and keloid) scars, hyperpigmented, and unsightly in appearance. Some factors influence the wound disfigurement, such as location, prolonged inflammation, infections, epithelialization delay, action of pericicatricial tissue, and tension forces of the adjacent skin.[1,3]

Aesthetic improvement of the scar

Studies in animals have shown that botulinum toxin type A injection in low doses could significantly improve the appearance of facial scars, as it would have an inhibitory action on the proliferation of the fibroblasts and, therefore, on the collagen production in a dose-dependent manner, reducing the appearance of retractions and improving the scar pattern. However, at high doses, it would have a negative effect by inhibiting the reepithelialization and decreasing the local angiogenesis.[1, 5, 6]

Also, the tension around the surgical scar is one of the most relevant factors in determining the final aesthetic outcome. This tension, in turn, is caused by the contraction of the local musculature. Usually, incisions are planned to follow the lines of force in parallel, but this is not always possible: thus it's interesting to use other techniques to reduce possible unaesthetic effects. Microtraumas caused by stress also induce prolonged inflammation, with increased metabolic activity and extracellular collagen and glycosaminoglycans deposition, leading to hypertrophic scars.[6 ,7, 8]

The application of botulinum toxin type A can be performed intraoperatively. One study demonstrated that the combination of botulinum toxin, anesthetic, and vasoconstrictor optimizes the botulinum toxin effect, resulting in earlier paralysis of the treated site. Botulinum toxin type B can also be applied, having a faster effect.[9]

Another study in primates observed, through standardized incisions, that the scars in the group treated with botulinum toxin had a positive esthetic result compared with the scars in the control group.[10] There is also in the literature a case report of patients who wished aesthetic improvement of a prior scar, performing a scar excision and intraoperative application of botulinum toxin, with a very satisfactory aesthetic result. Within three days, the surrounding musculature was paralyzed with minimal tension and distention from the wound margin.[6, 9] In another study, analyzing the thyroidectomy procedure scars of 30 patients, individuals in the group that received local botulinum toxin injection were more satisfied with the results than those that received saline injections. The scars treated with botulinum toxin type A became narrower, with natural coloring, and better overall appearance in the six months follow-up.[11]

Hypertrophic scar and keloids

Keloids and hypertrophic scars (HS) are caused by hyper-

proliferation of fibroblasts, resulting in excess collagen deposition. They are disfiguring and are often associated with clinical symptoms such as pruritus, pain, limited range of motion, contracture, and psychological effects on patients. Conventional options for the treatment of hypertrophic scars and keloids include intralesional injections of corticosteroids and 5-fluorouracil, surgery, cryotherapy, radiotherapy, laser therapy, and topical silicone gel sheets application. Recently, the use of botulinum toxin has been studied to treat symptoms and to prevent the formation of keloid and hypertrophic scars.[1, 12]

A recent study has reviewed the literature related to the subject and concluded that published clinical trials showed promising results demonstrating that botulinum toxin type A can modulate the development of keloids and hypertrophic scars. There appears to be scientific evidence that botulinum toxin type A negatively regulates TGF-b expression, reducing fibroblast proliferation and modulating collagen activity in pathological healing. However, keloid scars appear to be more resistant than hypertrophic scars to botulinum toxin type A therapy. Nevertheless, further studies are needed to more objectively define the applicability of this therapy.[12, 13, 14, 15]

Microdoses of botulinum toxin

The "microbotox" technique or microdoses of botulinum toxin application was developed by Wu in 2000 to provide more natural effects to patients. It is based on multi-point injection of highly diluted botulinum toxin every 0.8-1.0 cm into the dermis or at the between the dermis and the superficial layer of facial muscles.[1]

The main applications of this technique are to improve the appearance of fine lines and wrinkles by acting on the superficial muscles that insert into the skin. The use of the highly diluted botulinum toxin in small amounts at each injection prevents diffusion to deeper muscles, preventing a more frozen expression. Also, it has the advantage of reducing sweat production and the activity of the sebaceous glands, improving the appearance of the skin.[1, 16, 17, 18]

Lower face and neck region: When botulinum toxin is applied in microdoses in the anatomical region of the platysma muscle, it is observed an improvement in the neck skin texture and a decrease in the activity of the superficial fibers of this muscle, creating a "lifting" effect of the jowl and jaw areas, as well as a better cervical and mentalis muscle contour. This technique is indicated for patients with mild flaccidity of the cervical region, i.e., with early signs of aging that do not yet have an indication of surgery.[16]

This technique uses 20 units of 1 ml of saline solution for the preparation of microdoses of botulinum toxin. Two to three syringes containing 1 ml of the solution are used, and of each 1 ml syringe, about 100 to 120 microinjections should be performed. For the injection, the patient should be placed in a semi-reclined position, with the chin raised, keeping the skin stretched. Bleaching of the skin with the formation of papule should be observed at the time of the injection. A slight resistance should be felt when pressing the plunger: if the solution is easily injected, the needle was probably inserted very deeply.[16]

"Accordion wrinkles": Patients with significant photoaging and loss of volume may develop multiple parallel fine lines of varying depth ranging from the orbital region to the cervical region, which lead to the aspect of "scratch face" or "accordion lines". These wrinkles are more apparent when smiling, but over time they can become static. Its treatment is challenging due to the superficiality and length of the lines. The technique of multiple superficial injections of highly diluted botulinum toxin with or without hyaluronic acid (for skin hydration) may be employed, with a maximum of 40 units per side recommended. The effect on treated patients was a significant improvement in "accordion wrinkles" and skin appearance.[17, 18]

The microdoses of botulinum toxin technique is a new tool that has proven to be effective in treating some wrinkles and facial aging. However, a qualified professional should perform it since there is a risk of inadvertently paralyzing the deeper musculature of the treated area.

Scrotal rejuvenation

Scrotal wrinkling can also be referred to as scrotal rugosum or cutis scrotum gyratum. Some patients have excessive scrotal wrinkling and feel embarrassed at the time of sexual intercourse. The contraction of the dartos muscle, a rugated fascial muscle of the scrotum, in response to cold temperatures or sexual intercourse may result in the accentuation of scrotal wrinkling. As the contraction of the dartos muscle is a contributing component to the etiology of scrotal wrinkling, the botulinum toxin injection can result in a smoother, less wrinkled skin surface. There is no well-defined application protocol on the scrotal rejuvenation technique with botulinum toxin.[19]

Muscle definition

With the evolution of the modalities of botulinum toxin application, its extrafacial use has been gaining prominence, mainly in Asia. Oriental women tend to have shorter legs and when there is hypertrophy of the gastrocnemius muscle, the feeling of "stubby" legs is increased, which is considered an obstacle to beauty for the aesthetic standards of Asians, who seek more elongated legs, unlike Westerners. Botulinum toxin has been used as a noninvasive way of achieving this goal. It is applied to the medial head of the gastrocnemius muscle, which is the most prominent and functionally redundant calf muscle.

Bogari *et al.* demonstrated, through a three-dimensional magnetic resonance imaging study, that the most effective technique consists of the botulinum toxin application in 48 points, distant about 2 cm apart, at a dose of 1.5 UI at each point. The technique results in an effective reduction of the circumference of this leg region, giving the impression of a more elongated lower limb. It is important to emphasize that this technique can be used in thin patients, where the calf diameter is primarily due to gastrocnemius hypertrophy. Also, it should be used with caution as it may result in gait abnormalities and fatigue after walking or running. In this technique, the muscle decreases to approxima-

tely half of its original volume after five to six months, returning to its original volume 10-12 months after the injection.[20]

If the individual avoids active exercise of the treated muscle, the return to muscle volume pre-treatment can be prevented. Clinical experience indicates that repeated injections over several years may also result in chronic muscular atrophy.[20, 21, 22]

Non-aesthetic uses

The main use of botulinum toxin in Dermatology is related to facial aesthetics. In recent years, however, the botulinum toxin use in dermatological diseases with good results has been observed.[1] Several diseases nowadays find in botulinum toxin a differentiated option, whose effectiveness is being increasingly studied and proven, enabling dermatologists to offer new therapeutic options to their patients.[1, 3]

Rosacea

Rosacea is an inflammatory skin disease, with chronic and relapsing evolution, which presents clinically persistent facial erythema, papules, pustules, telangiectasias, and recurrent flushing. Its symptoms can cause embarrassment, low self-esteem, anxiety, worsening the quality of life of patients. Its treatment is challenging because, in general, symptoms respond only partially to traditional therapies, and the tendency for recurrence is significant. Given this picture, the use of intradermal botulinum toxin has been investigated as a new therapy. It would block the release of the neurotransmitter acetylcholine from the periphery of the nerves with reduction of cutaneous vasodilation and consequent reduction of facial erythema and flushing.[23, 24, 25]

Many studies have been published showing the benefits of botulinum toxin in rosacea treatment. One of the most recent studies assessed two Korean women with resistant symptoms of erythema and facial flushing and obtained satisfactory results. For the treatment, 50 UI of botulinum toxin was diluted in 2.5 ml of sterile saline, resulting in a concentration of 2 UI for every 0.1 ml of the solution. Intradermal botulinum toxin injections were applied vertically at a 90° angle at marked points every 1 cm in the entire erythema area, exceeding 1 cm of this area. Two sessions were held, with intervals of one week between them. In the first session, most of the product was applied, and in the second, it was applied only in the areas of remaining erythema.[25]

The mesotherapy is another form of botulinum toxin application, also presenting positive results. Bharti *et al.* described the intradermal botulinum toxin injection (10 U/mL) as 0.05 mL microdroplets in the central region of the face. The injections are spaced every 0.5 cm and applied under topical anesthesia. Improvement in erythema, edema, telangiectasia, and flushing was observed within one to two weeks, and this improvement lasted approximately three to four months when it was necessary to repeat the treatment.[26]

In 2017, Dayan *et al.* conducted a randomized double-blind study in which patients receiving botulinum toxin injections presented a significant reduction in the primary characteristics of rosacea at four weeks post-treatment, while the group receiving the same volume of injection with saline solution showed no improvement.[27] These new findings demonstrate that botulinum toxin can be considered a safe and effective agent to reduce the severity and symptoms of rosacea, in addition to increasing patient satisfaction.

Decreased acne/ sebum production

In recent years, several studies have shown promising results of the botulinum toxin use to improve skin oiliness and, consequently, acne. The results of recent publications have indicated a significant reduction in sebum production and have demonstrated a correlation between sebum production and injection techniques, although the dosage used is unknown. There is also evidence to suggest that the action of acetylcholine on the muscarinic receptor is an important regulator of sebum production.[1]

Shah published one of the first reports in the literature in 2008 where 20 patients were evaluated after the intradermal administration of botulinum toxin in the "T zone". A significant photographic improvement after one month of treatment was observed in skin oiliness, as well as a decrease in pore size in 17 of the 20 patients analyzed.[28]

Subsequently, Rose & Goldberg applied intradermal botulinum toxin in the frontal region of 25 patients, obtaining results that suggest that botulinum toxin reduces the sebum production in patients with oily skin, with a high degree of satisfaction.[29]

In 2015, Min *et al.* conducted the first prospective, randomized, double-blind study in 42 female patients to assess the amount of sebum in the frontal region of individuals treated with intramuscular botulinum toxin. In fact, the study confirmed the reduction of sebum excretion around the region of application of 2 UI of botulinum toxin without the benefit of larger doses (4UI). However, away from the injection site, the sebum production gradually normalized, even increasing in areas without botulinum toxin, perhaps by a compensatory mechanism. Sebum production was recovered after 16 weeks of follow-up. Additionally, although it was not the objective of the study, a reduction in the pore size in these patients was also observed.[30]

Craniofacial hyperhidrosis

Hyperhidrosis is defined as excessive sweating, being a common symptom in the population, and causing psychological and social problems. Craniofacial hyperhidrosis can affect only the face and scalp, or be part of a genetically engineered hyperhidrosis, often involving multiple sites of the skin. Botulinum toxin type A is well established in the treatment of hyperhidrosis in other sites. In the case of craniofacial hyperhidrosis, botulinum toxin type B, which is not widely used for this purpose, could be advantageous, as it would act less on motor neurons, thus preventing eyebrows hair loss.[31]

Karlgvist *et al.* used botulinum toxin type B to treat facial and scalp hyperhidrosis in 42 patients, finding a positive result in the reduction of sweat production and mainly in patients' quality of life. The treatment used 5 UI of intradermal botulinum toxin type B injection, spaced 15 mm between the points of applica-

tion throughout the hyperhidrosis area, sparing the forehead area distant less than 4 cm from the eyebrow. Of the treated patients, 18% complained of temporary forehead stiffness and eyebrows hair loss; nevertheless, most of these patients returned later for new applications due to the hyperhidrosis improvement.[31]

Another study reported the use of botulinum toxin type A in 11 female patients with postmenopausal craniofacial hyperhidrosis. Intradermal botulinum toxin injections of 0.1 ml (25 UI/ml concentration) were performed in the areas to be treated and 64% of the patients perceived complete response in their symptoms with no observed adverse events.[32]

Fox-Fordyce disease

Nowadays, given its already established use and its new applicability, botulinum toxin has been considered a therapeutic possibility for other skin diseases. Fox-Fordyce disease is characterized by intensely pruritic papules in the regions of the apocrine glands, for which there is currently no definitive treatment or a known cure. In 2016, a 52-year-old female patient with this disease was treated with intradermal botulinum toxin type A injections, 2 UI, every 2 cm, totaling 100 UI for both armpits. After the treatment, the patient presented a marked reduction in the size and number of axillary papules, as well as a complete improvement of the local pruritus. This case showed that botulinum toxin type A might be considered a therapeutic option for recalcitrant Fox-Fordyce disease. However, clinical trials are still necessary to assess the best treatment modalities for this disease.[33]

Hailey-Hailey's Disease

Hailey-Hailey's disease (HHD), also known as familial benign chronic pemphigus, is an unusual bullous dermatosis whose anatomopathological feature is characterized by suprabasal acantholysis, giving the epidermis the appearance of a "dilapidated brick wall". Clinically, it presents flaccid blisters, painful erosions, and fissures in the intertriginous regions, especially in the axillary and inguinal areas. The disease has a recurrent course and is often complicated by a secondary infection. Traditional treatments for Hailey-Hailey's disease include oral and topical antibiotics and corticosteroids, cyclosporin, dapsone, and methotrexate for patients with recalcitrant disease. However, none of these agents provides long-term relief for most patients, urging to seek other therapies. As Hailey-Hailey's disease is exacerbated by sweat, friction, and heat, botulinum toxin type A has been identified as useful for the treatment of this disease in the recent literature, since its injections result in denervation of the sweat glands, with reduction of sweating and less chance of maceration, consequently preventing the development of secondary infections.[1, 34, 35, 36]

Studies have shown that the use of botulinum toxin type A resulted in marked improvement and long-term remission of the disease, with the advantage of being easy to apply and having few adverse events. Some authors even suggest that currently it could be considered the first line of treatment after failure with topical corticosteroids and antimicrobials use.[34, 35, 36] It is recommended to use 100 IU to 200 IU of botulinum toxin type A in the affected sites, with an average of approximately 50 IU per armpit or groin. A 100 UI vial of botulinum toxin can be reconstituted in 4 mL of saline, reaching a dilution of 2.5 UI for every 0.1 mL of solution.[35]

Psoriasis

Inverse psoriasis affects especially the flexural areas, and is characterized by erythematous, exulcerated, and infiltrated plaques associated with local burning sensation and pruritus. Some studies and case reports have shown that botulinum toxin could be a therapeutic option for inverse psoriasis by reducing local sweating and, consequently, maceration and infection. Also, it was believed that it would inhibit neuropeptides, reducing inflammation and pain transmission.[1,2]

Zanchi *et al*. demonstrated the first favorable results with botulinum toxin use in a study with 15 patients with inverse psoriasis who were treated with injections of 2.4 UI of botulinum toxin, placed 2.8 cm apart from each other, totalizing 50 UI or 100 UI of toxin per patient. Improvement in erythema extent and infiltration intensity was observed in 87% of patients and maintained for 12 weeks after treatment. Due to these findings, because it acts in controlling inflammation and substances involved in the mechanism of inverse psoriasis, botulinum toxin may become a new option in inverse psoriasis treatment.[37]

In the treatment of plaque psoriasis, studies show less positive results. Bagherani *et al*. conducted the first double-blind randomized study to assess the efficacy of botulinum toxin in the treatment of plaque psoriasis. Twenty subjects were recruited, from which two psoriasis plaques were selected: in one, a saline solution was applied, and in the other, a total of nine injections of 4 UI of botulinum toxin type A was applied at each point. The treated plaques were reassessed after one, three, four and eight weeks of treatment but did not achieve a statistically significant improvement over the control group.[38] However, in our review, we found a case report demonstrating a sustained local improvement of a psoriasis plaque in a patient after a single injection of intradermal botulinum toxin.[39]

Thus, while the results, for the time being, are discouraging, further studies on the subject may still be necessary.

Notalgia paraesthetica

It is known that botulinum toxin inhibits the presynaptic release of acetylcholine and that acetylcholine mediates pruritus in atopic dermatitis. Additionally, the toxin also inhibits substance P and glutamate, probably involved in pruritus. Thus, botulinum toxin stands out as a possible therapeutic option for pruritic conditions.[40]

Notalgia paraesthetica is chronic sensory neuropathy that affects the interscapular area, characterized by local pruritus and hyperpigmentation of the region. Other associated symptoms are pain, paresthesia, hypoesthesia, hyperesthesia, and burning sensation. Usual treatments for this condition include local anesthesia, topical corticosteroids, and capsaicin. However, none of these shows good results and long-term efficacy.[41]

In 2007, Weinfeld proposed that botulinum toxin would

be an effective and safe treatment for notalgia paraesthetica. The author performed intradermal botulinum toxin injections of 4UI in the affected area, placed 2 cm apart from each other, in two patients followed for 18 months. They showed a significant improvement in local pruritus and hyperpigmentation in the long term.[41] Wallengren and Bartosik also reported improvement in pruritus in four patients with notalgia paraesthetica treated with botulinum toxin.[40]

On the other hand, Pérez *et al.*, in treating five patients with notalgia paraesthetica, found that improvement in pruritus varied in each case, but that no patient presented a complete resolution of the condition or improvement of the brownish spot.[42] Maari *et al.* also showed no improvement of pruritus and hyperpigmentation in notalgia paraesthetica when comparing treatment with botulinum toxin type A (10 patients) and placebo (10 patients).[43] Therefore, due to divergences in the literature, the benefits of botulinum toxin for this condition are debatable, requiring further studies.

Postherpetic neuralgia and other pain syndromes

Postherpetic neuralgia (PHN) is one of the most formidable adverse events of herpes zoster, which mainly affects elderly and immunocompromised populations. Neuralgia can be explained by the increase in the number of P nerve fibers at the site of infection and reduction in the number of large nerve fibers responsible for inhibiting the painful stimulus.[44, 45] The mechanism of action of botulinum toxin in pain relief is not fully understood; however it is believed to act by inhibiting the release of pain mediators, such as glutamate, substance P, and calcitonin gene-related peptide in the dorsal root ganglia, reducing inflammation around the nerve endings, deactivating the sodium channels, and inhibiting axonal transport.[46, 45]

One study investigated the effect of botulinum toxin on 58 patients presenting postherpetic neuralgia symptoms for four to 15 months. In this study, botulinum toxin was effective in reducing pain in 18 (31%) cases and showed significant results in 27 (46.6%) cases. However, it was ineffective in the remaining 13 (22.4%) patients. The severity of pain, frequency, and duration of events was significantly reduced after treatment.[47]

In a recent review study, level A evidence (effective) was observed not only for postherpetic neuralgia treatment but also for trigeminal and posttraumatic neuralgia.[48] Fischoff *et al.* concluded in their review that there is a moderate level of evidence on the efficacy of the botulinum toxin use for the treatment of trigeminal neuralgia and postherpetic neuralgia.[49]

Emad *et al.* assessed the efficacy of botulinum toxin in 15 patients with postherpetic neuralgia by injecting 15 IU per 10 cm^2 of affected area, obtaining improvement of pain in all patients, although the analgesic effect diminished over the weeks.[44] Xiao *et al.* compared the use of botulinum toxin over placebo and achieved a significant improvement in pain and sleep in patients treated with the toxin presenting symptoms of postherpetic neuralgia.[46] Another study, assessing 30 patients, also showed the efficacy of botulinum toxin over placebo in postherpetic neuralgia treatment, as well as its tolerability and safety.[50]

The application should be performed at points in the area of pain delimited by the patient, distant 1cm between each other, in the amount of 0.5-1 IU per point.

Despite the current advancement of treatments, postherpetic neuralgia persists in many individuals influencing their daily activities and reducing their quality of life. Many commonly used treatments present adverse events and should be used with caution in the elderly or in patients with multiple comorbidities. In this context, botulinum toxin presents as an interesting therapeutic alternative.

Raynaud's Disease

Raynaud's disease, or Raynaud's phenomenon (RP) is a vasospastic disorder of digital vessels, triggered by exposure to cold or stress. It is more commonly observed in the hands, but it also frequently affects the toes.[51] Botulinum toxin has been presented as a treatment modality for Raynaud's disease, being increasingly studied.[52]

In a review study on the use of botulinum toxin in Raynaud's disease, Zhou *et al.* concluded that botulinum toxin acts on vascular smooth muscle blocking norepinephrine transmission and preventing vasoconstriction, in addition to blocking alpha-adrenergic receptors leading to the reduction of cold-induced vasoconstriction, as well as pain.[52]

Dhaliwak *et al.* demonstrated the treatment of three patients with severe Raynaud's disease in the feet treated with 10 UI of botulinum toxin injected at the base of each toe, evolving with improved cold tolerance, color change, frequency and severity of the episodes six weeks after the application, with a duration of about five months.[51]

Patients with digital ulcers resulting from severe cases of Raynaud's disease may also benefit from the use of botulinum toxin. Studies have shown, through angiography, improved blood flow, with scarring of ulcers after the application of toxin.[53, 54]

Bello *et al.* performed a double-blind, controlled, clinical trial with scleroderma patients who received botulinum toxin applications in one hand and saline solution in the other. The study concluded that there should be some positive effect, but it was questionable as to its significance.[51]

The role of botulinum toxin in the treatment of Raynaud's disease should be more studied in populations of more homogeneous patients and in unique clinical situations, such as acute digital ischemia, to better elucidate its real efficacy and indication.[55]

Treatment of recalcitrant chronic pruritus

Localized chronic pruritus is a common symptom that significantly affects health and quality of life. Botulinum toxin showed potential as an antipruritic agent in patients with localized chronic pruritus, refractory to conventional therapies.[56]

Pruritus is driven by C nerve fibers, which are sensitive to neurotransmitters, histamine, and other inflammatory mediators, such as substance P and calcitonin gene-related peptide. A recent study showed that botulinum toxin is responsible for

reducing histamine-induced pruritus, as well as vasomotor reactions and neurogenic inflammation.[56] Other studies have shown that botulinum toxin reduces the release of glutamate, substance P, and calcitonin gene-related peptide.[57] Some studies indicate that botulinum toxin is an effective option in the treatment of lichen simplex chronicus, since pruritus is marked in this pathology.[58]

As the number of studies is limited, there is still insufficient evidence to reach a conclusion on the effectiveness of botulinum toxin in the treatment of chronic pruritus.

CONCLUSION

Botulinum is a simple, safe method with satisfactory results and is well established throughout the world for the treatment of facial wrinkles. Ongoing research is identifying the use of botulinum toxin for other purposes, both in the aesthetic and in the therapeutic field. This study aimed at conducting a literature review on these applications, possibly useful in the dermatologist practice.

In the aesthetic area, botulinum toxin has shown benefit in the treatment of hypertrophic scars, scrotal rejuvenation, definition of the gastrocnemius muscle, and in microdoses in the face and neck region. However, for the treatment and prevention of keloids, further studies are still necessary.

Moreover, in the treatment of dermatological disorders, this review showed that botulinum toxin might be useful for postherpetic neuralgia and other pain syndromes, craniofacial hyperhidrosis, rosacea, and Hailey-Hailey's disease.

Also, new studies show that its use could be extended to other diseases such as recalcitrant chronic pruritus, Raynaud's disease, notalgia paraesthetica, inverse psoriasis, and Fox Fordyce disease. However, clinical trials are necessary before these treatments become a clinical reality.[1]●

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The association between *Staphylococcus* epidermidis and palmitic acid level in patients with acne vulgaris

Associação entre Staphylococcus *epidermidis e o nível de ácido palmítico em pacientes com acne vulgar*

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ABSTRACT

Introduction: Acne vulgaris is an inflammatory disorder of the pilosebaceous gland. Palmitic acid is one of the major types of free fatty acid and may play a role in acne pathogenesis. In addition, recent studies suggested that Staphylococcus epidermidis might be involved in acne.

Objective: To explore the association between Staphylococcus epidermidis and palmitic acid in acne vulgaris.

Methods: Forty-three high school students at an urban area in South Sulawesi, Indonesia, were included. The palmitic acid level was measured using gas chromatography and comedone was cultured to detect the microbiota profile. Mann-Whitney test was used to analyze the median palmitic level difference between groups with different acne vulgaris severity.

Results: Fourteen patients (32.6%) had mild acne vulgaris, while 14 and 15 patients had moderate and severe acne vulgaris, respectively. The severe and moderate group showed significantly higher palmitic acid level compared with the mild group. Subgroup analysis of patients with moderate and severe acne vulgaris positive for S. epidermidis showed a significantly higher palmitic acid level compared with the mild group.

Conclusions: This result suggests that S. epidermidis may be associated with the palmitic acid level and may contribute to acne pathogenesis.

Keywords: Acne vulgaris; Staphylococcus epidermidis; Palmitic acid

RESUMO

Introdução: *A acne vulgar é um distúrbio inflamatório da glândula pilossebácea. O ácido palmítico é um dos principais tipos de ácidos graxos livres e pode desempenhar um papel na patogênese da acne. Além disso, estudos recentes sugeriram que o Staphylococcus epidermidis pode estar envolvido na acne.*

Objetivo: *Explorar a associação entre a Staphylococcus epidermidis e o ácido palmítico na acne vulgar.*

Métodos: *43 estudantes do ensino médio de uma área urbana do sul de Sulawesi, na Indonésia, foram incluídos. O nível de ácido palmítico foi medido utilizando cromatografia gasosa e os comedões foram cultivados para detectar o perfil do microbioma. O teste de Mann-Whitney foi utilizado para analisar a diferença do nível palmítico médio entre os grupos com diferentes graus de gravidade da acne vulgar.*

Resultados: *14 pacientes (32,6%) apresentavam acne vulgar leve, enquanto 14 e 15 pacientes apresentavam acne vulgar moderada e grave, respectivamente. O grupo grave e moderado apresentou nível de ácido palmítico significativamente maior em comparação ao grupo leve. A análise de subgrupo de pacientes com acne vulgar moderada e grave, positiva para S. epidermidis, mostrou um nível significativamente maior de ácido palmítico comparado ao grupo leve.*

Conclusões: *Esses resultados sugerem que S. epidermidis pode estar associado ao nível de ácido palmítico e pode contribuir na patogênese da acne.*

Palavras-Chave: *Acne vulgar; Staphylococcus epidermidis; Ácido palmítico*

Original Articles

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INTRODUCTION

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous glands where lesions can be non-inflammatory (open and closed comedones) or inflammatory (papules and pustules).[1,2] Acne vulgaris mostly occurs in adolescence to young adulthood and can cause post-inflammatory hyperpigmentation and scarring. A study conducted by Bhate *et al* showed that acne can be found in about 20% of young adults.[3] In addition, acne has a high persistence in which 43% of those over 30 years still have acne. Acne also has a strong genetic predisposition in which 80% of acne cases can be inherited to close relatives.[3,4]

The human skin hosts a diverse group of microorganisms in which *Staphylococcus*, *Cornybacterium*, and *Propionibacterium* constitute 60% of all bacterial species.[5] However, there are only limited studies examining the entire microbiota population in patients with acne and their role in acne pathophysiology. Despite the widely accepted view of the role of *Propionibacterium acnes* (*P. acnes*) in triggering acne, recent studies have suggested that *Staphylococcus epidermidis* (*S. epidermidis*) may also be involved in acne pathogenesis. Pathak *et al*.[6] showed that the microbial load of *S. epidermidis* increases in acne lesions compared with control. This result is in line with a recent study where *S. epidermidis* was found in a more abundant manner compared to *P. acne*.[7], suggesting the possible role of *S. epidermidis* in acne pathogenesis.

Besides microbiome, free fatty acid (FFA) has also been shown to be involved in the development of acne as increased level of FFA will lead to neutrophils influx through chemotaxis process.[8] A high-fat diet has been shown to induce or aggravate acne vulgaris lesions.[9] Of all FFA types, palmitic acid and its derivatives have been shown to be the most abundant type.[10-12] Palmitic acid may stimulate the release of various proinflammatory cytokines and contribute to pilosebaceous duct hyperkeratinization and acne inflammation.[13]

To the best of our knowledge, no study has observed the association between *S. epidermidis* and palmitic acid and their association with acne vulgaris. The aim of this study is to investigate the microbiome profile, especially *S. epidermidis*, in patients with acne vulgaris and to investigate its association with palmitic acid level in inducing acne vulgaris lesions.

METHODS

This cross-sectional study was conducted in a high school in Makassar, South Sulawesi, Indonesia, from July to August 2017. The subjects were given explanation about the study and those who agreed were asked to sign an informed consent form (Ref. Number; 145/H4.8.4.5.31/PP36-KOMETIK/2017 from Hasanuddin University Ethics Committee). Subjects who did not take retinoids, antibiotics, anti-inflammatory drugs, and other anti-microbial products, such as antifungal soap and shampoo, in the last one month were assessed for acne severity with the Lehman Criteria and classified into mild, moderate, or severe acne vulgaris.[14] They were also given questionnaires to record family history of acne and dietary consumption.

Sebum was extracted from the forehead, nasolabial, cheek, and chins by an absorbent paper using acetone and diethyl ether with a ratio of 1:1 and then methylated using 0.2 M phenyltrimethylamine hydroxide solution in methanol. Gas chromatography was used to examine the product. The standard reference used was Supelco® 37 component FAME Mix. The standard concentration used for palmitic acid was 601 ppm injected into the gas chromatograph. Analysis was performed on a GC-MS QP 2010 Ultra Shimadzu Autosampler with a splitless injector. Separations were achieved using SH-Rxi-5Sil MS capillary column (30m x 0.25mm). Helium was used as the carrier gas at flow rates of 1.99mL/min and a split less ratio of 1:10. The injector temperature was 250^{0}C. The oven temperature was programmed at 140^{0}C for a hold of 10 minutes and increased to 250^{0}C with a flow of 7^0 C/min. It was further held for 10 minutes, resulting in a total analysis time of 35.71 minutes. MS spectra were obtained at range width m/z 40-500m/z, ion source and interface temperature 210^{0}C and 255^{0}C, respectively, and solvent cut time 3 minutes.

Sample from comedonal lesions was taken using a sterile comedone extractor and cultured in blood agar which was incubated at 37^{0}C for 48-72 hours. The growing colonies were observed under the microscope with gram staining. Each colony was made into a suspension equivalent to McFarland 0.5. The gram-positive and the gram-negative suspensions were taken 145µl and 280µl, respectively, and added into reaction tubes containing 3 ml saline solution. The gram-positive suspension was put into GPcard and AST GP 67 cards and the gram-negative suspension was put into GNcard dan ASTN 100 cards before final analysis using Vitek®.

Data analysis was done by using Statistical Package for Social Sciences (SPSS) 18.0 for Windows (SPSS Inc. Chicago, IL, USA). Mann-Whitney test was used to analyze the median palmitic level difference between groups with different acne vulgaris severity. A p-value <0.05 was considered significant.

RESULTS

The demographic data in Table 1 shows that of 43 participants, most participants (33 patients, 76.7%) were men and adolescent (15.77 $\pm$ 0.84 years). There were 25 participants with a positive family history of acne vulgaris (58.1%). As for the disease duration, 34.8% of the participants (15 subjects) have been having acne for 2 years and 10 subjects (23.3%) had had acne for 1 year and 3 years, respectively. Most of the patients (31 subjects, 72.1%) consumed fatty foods. The population showed almost uniform acne severity distribution: 14 patients were diagnosed with mild acne, 14 patients with moderate, and 15 patients with severe acne.

Figure 1 shows the concentration of palmitic acid in severe acne (median = 30,400 ppm) was significantly higher than that in the mild acne (30,400ppm vs 12,746ppm). The palmitic acid level in moderate acne was also found to be significantly higher than in the mild acne ($p<0.05$). However, no difference in palmitic acid level was found between patients with moderate and severe acne.

Table 2 shows the frequency of the microorganism found in the participants. It was shown that *S. epidermidis* was the most common microorganism cultured (32 subjects, 74.41%) and while non-*S. epidermidis organisms* were isolated in 11 cases (25.59%). The isolated non-*S.epidermidis* microorganisms were *Staphylococcus hominis* (4 cases), *Staphylococcus warneri* (2 cases) *Staphylococcus xylosus* (1 case), *Staphylococcus aureus* (1 case), *Staphylococcus haemolyticus* (1 case), *Staphylococcus capitis* (1 case), *Lactobacillus plantarum* (1 case).

Data in Figure 2 shows that there was no association between *S. epidermidis* and palmitic acid level. However, the data in Figure 3 on subgroup analysis of patients with positive *S. epidermidis* culture showed that palmitic acid level in subjects with moderate acne vulgaris was significantly higher compared with mild acne vulgaris (p=0.006). Furthermore, subjects with severe acne vulgaris were also found to have higher palmitic acid level compared with those with mild acne with a borderline significance level (p=0.064).

DISCUSSION

Data from our study showed that higher palmitic acid level indicated a greater degree of acne severity. Palmitic acid levels in the facial skin of patients with severe and moderate acne vulgaris were found to be significantly higher than those with a mild degree of acne vulgaris. Several studies have suggested the role of palmitic acid in inducing pro-inflammatory

Table 1: Basic characteristics of the population

Category	Frequency (n)	Percentage
Gender		
Man	33	76,7
Women	10	23,3
Age (average,SD)	15,77 ± 0,84 anos	
Family History		
Present	25	58,1
Absent	18	41,9
Food		
Milk	7	16,3
Chocolate	3	7,0
Fatty foods	31	72,1
High Sugar Food	2	4,7
Acne Severity Degree		
Light	14	32,6
Medium	14	32,6
Weight	15	34,9
FFA level ppm (median, IQR)	24,358 (12,946-39,838)	
PCR to P. acnes		
Positive (+)	5	11,6
Negative (-)	38	88,4

Fonte: Tabri F, *et al.* 2018[15]

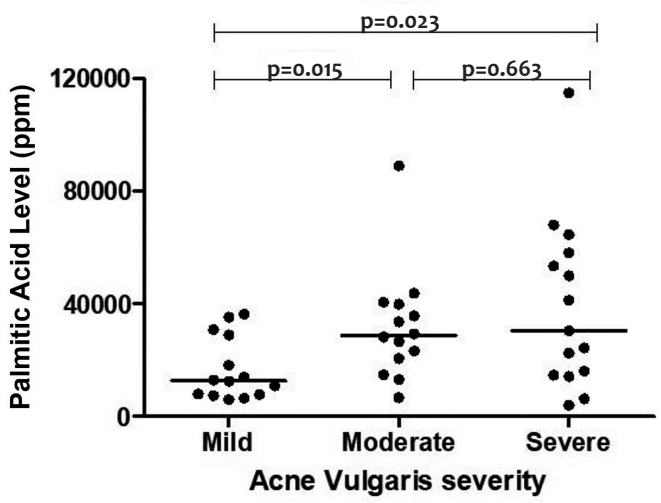

Figure 1: Comparison of palmitic acid levels between different acne groups

Source: Tabri F, *et al.* 2018[15]

Table 2: Frequency of microorganisms found in participants

Etiology	Frequency (n)	Percentage (%)
Staphyloccocus epidermidis	32	74.41
Non-Staphyloccocus epidermidis	11	25,59%
Staphylococcus hominis	4	
Staphylococcus warneri	2	
Staphyloccocus xylosus	1	
Staphyloccous aureus	1	
Staphylococcus haemolyticus	1	
Staphylococcus capitis	1	
Lactobacil/us plantarum	1	
Total	43	100

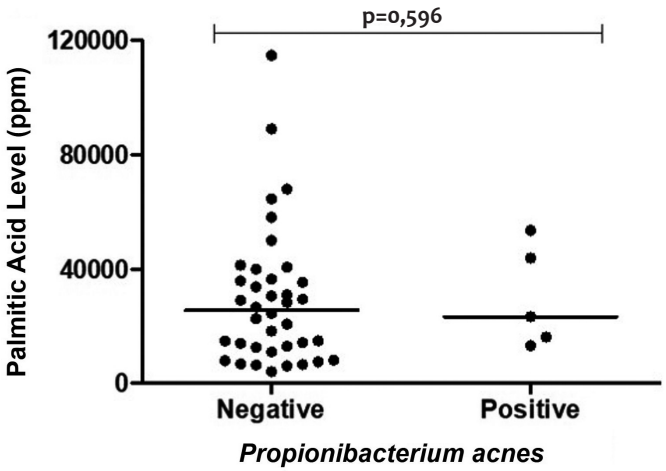

Figure 2: Association between palmitic acid and P. acnes levels, identified by PCR

Source: Tabri F, *et al.* 2018[15]

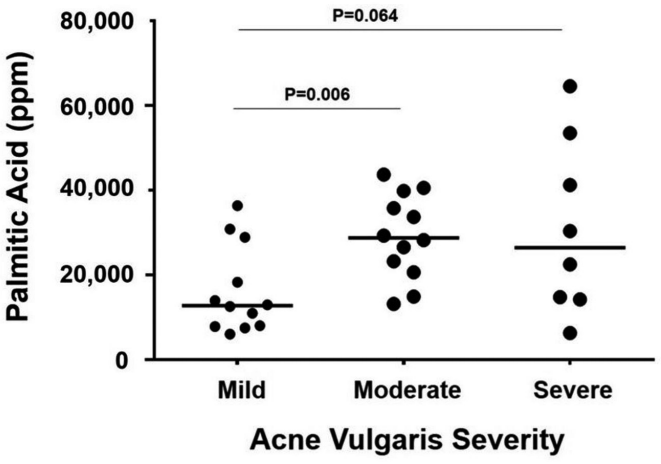

FIGURE 3: Analysis of the association between acne vulgaris and palmitic acid in the subgroups

cytokines, which contributes to inflammation and pilosebaceous duct hyperkeratinization.[13, 15] Palmitic acid was shown to inhibit hydrogen peroxide which caused proinflammatory mediators to pass more easily to the dermis and aggravate inflammation.[15]

Data in table 2 showed that *Staphylococcus epidermidis* was the most common microorganism found in the subjects. Interestingly, there was no *P. acne* obtained through culture, despite the classical view that *P. acne* induces acne. Dreno *et al.*[7] found a similar result, where *S. epidermidis* was the most abundant bacteria found in patients with acne vulgaris. They also observed that *P. acne* only constitutes less than 2% of the whole population. In contrast, other studies showed that in normal population, *P. acne* may represent up to 30% of the whole facial microbiota.[16, 17] One possible explanation for this finding is that the aerobic and facultative anaerobic *S. epidermidis* mediates fermentation process which inhibits the growth of the anaerobic *P. acne*.[18]

Although the data in figure 2 shows that *S. epidermidis* was not associated with palmitic acid level, subgroup analysis of patients with positive *S. epidermidis* culture interestingly showed that the palmitic acid level in patients with moderate acne vulgaris to be significantly higher compared with those with mild disease (p=0.006). Those with severe acne were also found to exhibit higher palmitic acid level compared with those presenting mild acne with borderline significance level (p=0.064). Studies have suggested the possible role of palmitic acid inducing acne vulgaris through the activation of mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway, triggering the activation of toll-like receptor 2 (TLR2), and pilosebaceous duct hyperkeratinization,[13, 19, 20] In addition, the expression of NLRP3 and inflammasome-mediated IL-1β production are also upregulated which further perpetuates the inflammation process.[21] Taken together, this result suggests that *S. epidermidis* may be associated with the level of palmitic acid level and hence be involved in the pathogenesis of acne vulgaris. Further biomolecular studies are needed to confirm this hypothesis.

Recent literature showed that *S. epidermidis* may be involved in the pathogenesis of acne vulgaris.[22] A study by Bialtecka *et al.* showed that *S.epidermidis* induces inflammation in the form of Nitric Oxide production within the infected tissue. They also induce macrophage activation, as shown by the increase of TNF-alpha production and IL-12 production after incubation with *S. epidermidis*.[23] This topic remains contentious, however, as *Staphylococcus* was shown to activate TLR2 and induce miR-143 in the keratinocytes that target 3' UTR of TLR2 and decrease the stability of TLR2 MRNA and TLR2 protein; resulting in the inhibition of *P. acne* proinflammatory cytokines.[24, 25] Also, a study has suggested that fatty acid composition did not associate with the presence of acne, although they might differ in accordance with the amount of triglyceride.[26] Furthermore, *S. epidermidis* is able to form biofilm by secreting the exopolysaccharide intercellular adhesion, which supports an anaerobic condition for P. acne to flourish.[27, 28] With the above-mentioned data, whether skin microbiome provides a protective or pathogenic effect remains debatable.[29]

To the best of our knowledge, this is the first study to explore the association between *S. epidermidis* and palmitic acid level and their effect in acne vulgaris. This is an initial study conducted to assess the prevalence of acne vulgaris in the adolescent population of an urban area and its association with palmitic acid levels patients with acne vulgaris. However, as a preliminary study, the sample size is limited and hence some subgroup analysis could be conducted. This study also only assessed one type of free fatty acid. Squalene and its peroxide, for example, has been thought to contribute to inducing inflammation and comedone formation.[30] Futures larger scale studies examining more than one type of free fatty acid need to be done. Nevertheless, this study provided important data and thus delivered a foundation for future studies in this field.

The result of this study shows that *S. epidermidis* is the most common microorganism cultured from the participants. In addition, *S. epidermidis* may be associated with higher palmitic acid levels which may contribute to the development of acne vulgaris. Further studies on the underlying mechanism of *S. epidermidis* in inducing palmitic acid level and acne vulgaris need to be conducted to confirm this hypothesis.

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Design and planning of the study, data collection, analysis and interpretation, preparation and writing of the manuscript, critical review of the manuscript. Approval of the final version of the manuscript.

Evaluation of insulin resistance and risk factors for cardiovascular diseases in patients with vitiligo

Avaliação de resistência à insulina e fatores de risco para doenças cardiovasculares em pacientes com vitiligo

Original Articles

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ABSTRACT

Introduction: The relationship between cardiovascular disease, insulin resistance and vitiligo has been evaluated in studies. However, there is still no consensus on the subject.

Objectives: To evaluate the relationship between insulin resistance and vitiligo, in addition to the prevalence of risk factors for cardiovascular disease, in adults with vitiligo when compared to the control group.

Methods: Cross-sectional study with a control group. A convenience sample of consecutive patients aged 14 years and over was used. Patients and controls were assessed with laboratory tests and anthropometric measurements. The LAP, HOMA-IR, and HOMAβ indices were calculated.

Results: We included 130 patients, 73 with a diagnosis of vitiligo and 57 controls. There were no significant differences between groups when LAP, HOMA-IR, and HOMAβ were evaluated. Among the risk factors for cardiovascular disease, systolic blood pressure was significantly higher in patients with vitiligo.

Conclusions: There was no higher prevalence of insulin resistance among patients with vitiligo. Regarding the risk factors for cardiovascular diseases, only systolic blood pressure was higher in patients with vitiligo. Further studies are needed to elucidate the prevalence of insulin resistance and cardiovascular risk factors in patients with vitiligo.

Keywords: Vitiligo; Skin diseases, Metabolism; Metabolic diseases; Insulin resistance

RESUMO

Introdução: *A relação entre doença cardiovascular, resistência à insulina e vitiligo tem sido avaliada em estudos. No entanto, ainda não há consenso sobre o assunto.*

Objetivos: *Avaliar a relação entre resistência à insulina e vitiligo, além da prevalência de fatores de risco para doença cardiovascular em adultos com vitiligo quando comparados ao grupo controle.*

Métodos: *Estudo transversal com grupo controle. Foi utilizada uma amostra de conveniência de pacientes consecutivos com 14 anos ou mais. Pacientes e controles foram investigados com exames laboratoriais e medidas antropométricas. Foram calculados os índices LAP, HOMA-IR, e HOMAβ.*

Resultados: *Foram incluídos 130 pacientes, 73 com diagnóstico de vitiligo e 57 controles. Não houve diferença significativa entre os grupos quando avaliadas as medidas do LAP, HOMA-IR, e HOMAβ. Dentre os fatores de risco para doença cardiovascular, a pressão arterial sistólica foi significativamente maior nos pacientes com vitiligo.*

Conclusões: *Não foi observada maior prevalência de resistência à insulina entre pacientes com vitiligo. Quanto aos fatores de risco para doenças cardiovasculares, apenas a pressão arterial sistólica foi maior nos pacientes com vitiligo. Novos estudos são necessários para elucidar a prevalência de resistência à insulina e fatores de risco cardiovascular em pacientes com vitiligo.*

Palavras-Chave: *Vitiligo; Doenças metabólicas; Doenças da pele, Metabolismo; Resistência à insulina*

INTRODUCTION

Vitiligo is an acquired multifactorial disease that is characterized by the appearance of macromolecules and achromatic spots on the skin and mucous membranes due to the disappearance of melanocytes in the affected areas.[1] According to data from regional studies worldwide, its prevalence can vary from 0.06% to 2.28% in adults and from 0 to 2.1% in children.[2] Studies show an increased prevalence of autoimmune diseases in vitiligo patients, such as Hashimoto's thyroiditis, type 1 diabetes mellitus, Addison's disease, alopecia areata, and pernicious anemia, among others.[3,4] In addition to these associations, the relationship between cardiovascular disease, insulin resistance (IR) and vitiligo has been studied. Some studies suggest a higher prevalence of type 2 diabetes in patients with vitiligo;[5] the only study that related the disease with IR, comparing patients with controls, found a higher prevalence of IR among patients with vitiligo.[6] However, another study that assessed diagnostic criteria for metabolic syndrome found a better metabolic profile in patients with vitiligo. Therefore, there is no consensus on the subject to date.[7]

The dysfunction of β-cells and IR are interrelated metabolic abnormalities in the etiology of type 2 diabetes mellitus (T2DM). IR is characterized by target cells failure to respond to normal levels of circulating insulin, resulting in compensatory hyperinsulinemia in an attempt to obtain an adequate physiological response. Due to the association between IR and endothelial dysfunction, an initial step for the atherosclerosis process, the disease has been considered as an independent predictor of cardiovascular disease.[8] The hyperinsulinemic-euglycemic clamp is the gold standard for evaluating IR, but difficult to perform. The Homeostasis Model Assessment Insulin Resistance (HOMA-IR) and Homeostasis Model Assessment β-Cell Function (HOMAβ) methods, which measure the functional capacity of β-pancreatic cells, were validated for the diagnosis of IR against the gold standard, supporting its use in epidemiological studies.[8,9]

LAP (lipid accumulation product) is a central lipids accumulation index based on a combination of waist circumference (WC) and triglycerides (TG). It was created to describe how an individual altered the path of abdominal waist growth and serum triglyceride levels in the Third National Health and Nutrition Examination Survey (NHANES III). The calculation formula includes the minimum WC values used to define specific points according to the patient's gender (65cm and 58cm for men and women, respectively). Current literature suggests a strong association between the lipid accumulation product (LAP) and metabolic syndrome.[10,11,12]

The objective of this study was to assess the relationship between IR and vitiligo by the HOMA-IR, HOMAβ and LAP methods, as well as to evaluate the cardiovascular risk factors in adults with vitiligo when compared with the control group.

METHODS

The sample size calculation was performed aiming to find a four times higher difference in the HOMA-IR variable than that found in the study by Karadag *et al.*,[6] which was 0.3, with a standard deviation of 2.6 in the cases and 1.2 in the controls. Considering a power of 80%, and a significance level of 5%, a total sample size of 110 subjects was reached, with 55 in each group. The program WinPepi, version 11.43 was used.

A cross-sectional study with the control group was conducted. We used a convenience sample of cases and controls with consecutive 14 years or more of patients in the Dermatology Outpatient Clinic of the Hospital de Clínicas de Porto Alegre. Patients with psoriasis, diabetes and using immunosuppressive drugs were excluded. Patients and controls were investigated with laboratory tests including insulin, C-peptide, fasting glycemia (FG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides (TG). Anamnesis and physical examination were performed, measuring waist circumference (WC), hip (HC), weight, height, and blood pressure (BP). From these data, HOMA-IR, HOMAβ, LAP indexes and waist-hip ratio (WC/HC) were calculated. The HOMAβ index was calculated by the formula (FG (mg/dL)) x (insulin (μUI/mL))/22.5, and HOMA-IR by the formula (20 x insulin (μUI/mL) mg/dL) - 3.5).[13]

The LAP index was calculated using the formula: (WC(cm) - 65) x (TG concentration (mmol/l)) for men, and (WC(cm) – 58) x (TG concentration (mmol/l)) for women.[12] The statistical analysis was performed in the SPSS 18.0 program. The categorical variables were compared by the chi-square test or Fisher's exact test. The quantitative variables with symmetrical distribution were compared between the groups by Student's t-test and described with mean ± standard deviation. Those with asymmetric distribution were compared by the Mann-Whitney test and described with median and interquartile range.

Covariance analysis (ANCOVA) was used to adjust the difference in the outcomes for potential confounding factors: age, BMI, dyslipidemia, hypertension, smoking, alcohol consumption, and antihypertensive use. A significance level of 5% was considered. The study was approved by the institution's ethics committee, and patients signed an informed consent.

RESULTS

A total of 130 patients were included, 73 with diagnosis of vitiligo and 57 controls; 65% of the patients were women in the vitiligo group and 70% in the control group. The mean age in the vitiligo group was 43 years, and 49 years in the control group (p=0.04). There was no statistical difference between the groups when gender, BMI, weight, hypertension diagnosis or antihypertensive use, cardiopathy, dyslipidemia, alcohol consumption or current smoking were assessed (Table 1). There were no significant differences between groups when LAP, HOMA-IR, HOMAβ, insulin, and C-peptide measures were compared, even when the variables were controlled for potential confounding factors. The groups were also not statistically different when the waist-hip ratio, HDL, LDL, LDL/HDL ratio, and TG were assessed. Mean systolic BP was significantly higher in the vitiligo group when compared with controls (124.57 ± 18.01 mmHg *versus* 121.19 ± 18.5 mmHg; p=0.01). The glycemia levels were

higher in the vitiligo group than in the control group (92.04 ± 10.01mg/dL *versus* 90.73 ± 9.92mg / dL), but without statistical significance (p = 0.07) (Table 2).

DISCUSSION

Different from our results, the only controlled study in adults with vitiligo that assessed IR, published by Karadag *et al.*, found significantly higher insulin resistance among vitiligo patients. This study evaluated 96 individuals, 57 patients with vitiligo and 39 individuals in the control group, all with similar age and body mass index. In fasting, insulin, C-peptide, glucose, CT, TG, LDL, HDL, and BP were collected. IR was calculated using the HOMA-IR assessment method. Patients with vitiligo had significantly higher levels of HOMA-IR, insulin, and C-peptide.[6] In our study, higher levels of insulin, C-peptide, and HOMA-IR were also found in the vitiligo group, but without statistical significance. It is not known, however, whether these differences in insulin levels between the groups found in these studies actually have any clinical impact, posing a greater risk of progression to T2DM or cardiovascular events. Longitudinal studies and larger sample sizes are needed to elucidate this question.

As in our study, the systolic blood pressure of patients with vitiligo was higher in comparison with the patients of the control group in the study cited above.[6]

The controlled study that addressed the prevalence of diagnostic criteria for metabolic syndrome in adult patients with vitiligo, published by Rodríguez-Martín *et al.*, found a better metabolic profile in patients with vitiligo. It included 105 patients with vitiligo and 95 in the control group and found a lower prevalence of altered levels of triglycerides and abdominal circumference in patients with vitiligo.[7] Also, there was a lower prevalence of altered levels of HDL in the vitiligo group, unlike the Karadag study, which found lower levels of HDL and a higher LDL/HDL ratio in patients with vitiligo.[6] The authors believe that this finding may have an enzymatic basis that results in a negative association between vitiligo and cardiovascular risk factors.[7]

The biological hypotheses used to explain the probable relationship between vitiligo, insulin resistance, and risk factors for cardiovascular disease are based on the likely dysfunction in the antioxidant mechanism of vitiligo patients.[14] Studies have shown a tendency to low activity of enzymatic and non-enzymatic antioxidants, such as catalase, glutathione peroxidase, and vitamin E, in patients with vitiligo, possibly increasing H2 O2 toxicity in affected tissues.[15,16] However, some studies found no difference in these markers when comparing vitiligo patients and controls.[17,18] The melanocytes production in adipose tissue would act as a protective factor against oxidative stress in this tissue, which could lead to greater oxidative stress in the adipose tissue of individuals with vitiligo, according to the study.[19] Another study found high levels of homocysteinemia in patients with vitiligo, which could be related to increased cardiovascular risk, according to authors.[20] However, the real influence of these markers on systemic oxidative stress is questioned, since to date the clinical studies have had conflicting results. As a limitation of the study, we can cite the cross-sectional design. To date, there are no studies with another design.

Table 1: Demographic data of vitiligo patients and controls

	Vitiligo (n=73)	Controls (n=57)	*p*
Women, n (%)	48 (65,8%)	40 (70,2%)	0.72
Age, mean ± SD, years	43.00±17.82	79.35±17.71	0.04
Weight, mean ± SD, kg	72.61±17.12	73.19±14.59	0.84
Body Mass Index, mean ± SD	27.00±5.45	27.16±4.75	0.86
Hypertension, n (%)	21 (28.8%)	17 (29.8%)	1
Use of antihypertensive, n (%)	20 (22.3%)	14 (24.6%)	0.95
Dyslipidemia, n (%)	9 (12.3%)	6 (10.7%)	0.99
Heart disease, n (%)	2 (2.3%)	5 (9.1%)	0.15
Smoking, n (%)	11 (15.1%)	10 (17.9 %)	0.32
Alcohol consumption, n (%)	6 (8.2%)	7(12.5%)	0.71

SD: standard deviation

Table 2: Comparison between anthropometric measures and exams between vitiligo patients and controls

	Vitiligo (n=73)[1]	Controls (n=57)[1]	*p**
Waist/hip ratio	0.85(±0.09)	0.85 (±0.06)	0.99
Systolic BP (mmHg)	124.57(±18.01)	121.19 (±18.50)	0.01
Diastolic BP (mmHg)	76.61(±11.14)	74.50 (±8.25)	0.11
Glycemia (mg/dL)	92.04 (±11.01)	90.73 (±9.92)	0.07
Total cholesterol (mg/dL)	194.28 (±41.36)	203.07 (±46.68)	0.63
HDL cholesterol (mg/dL)	49.58 (±14.15)	49.91 (±12.33)	0.92
LDL cholesterol (mg/dL)	122.04 (±32.72)	129.65 (±43.59)	0.51
LDL/HDL ratio	2.61 (±0.88)	2.75(±1.17)	0.56
Triglycerides (mg/dL)	95.0(76.5-130.5)	99.0(76.5-143.0)	0.94
Insulin (µU/mL)	10.56 (7.55-16.23)	9.71 (7.24-15.70)	0.43
C-peptide (ng/mL)	1.46(1.18-2.21)	1.45(1.12-2.13)	0.51
HOMA IR	2.47(1.68-3.71)	2.11(1.64-3.66)	0.27
HOMA beta	156.16(101.16-211.13)	137.7(101.87-237.27)	0.49
LAP	2787(1395-4278)	3038(1675-4648)	0.8

1. Quantitative variables with symmetrical distribution: mean ± standard deviation, asymmetric distribution: median and interquartile range.

* Control for variables of BMI, alcohol consumption, smoking, dyslipidemia, hypertension, age and antihypertensive use.

CONCLUSIONS

Our results showed no difference between groups when assessed for IR. Among the risk factors for cardiovascular diseases, only systolic BP was higher in the vitiligo group. Therefore, we cannot affirm that patients with vitiligo present a poorer metabolic profile or higher IR when compared with controls.

Further studies are needed to evaluate the relationship between vitiligo, IR, and cardiovascular risk in order to elucidate the importance of these factors in the evolution of the disease, which is of great relevance for the clinical management of patients.

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Original Articles

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Treatment of idiopathic guttate hypomelanosis with Er:YAG 2940nm laser alone or combined to piperine drug delivery: a pilot and comparative study

Tratamento da hipomelanose gutata idiopática com laser Er:YAG 2940nm isolado ou combinado ao drug delivery de piperina: um estudo-piloto comparativo

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ABSTRACT

Introduction: Idiopathic guttate hypomelanosis is an acquired leukoderma, whose available therapeutic options have inconsistent results.

Objectives: To evaluate the effect of isolated or combined ablative fractionated laser to drug delivery of piperine for treatment of idiopathic guttate hypomelanosis.

Materials and methods: In a prospective and comparative study, seven patients with idiopathic guttate hypomelanosis in the legs were elected, and five lesions were selected on each leg of each. Both legs of each patient were submitted to two Er:YAG 2940nm laser sessions, followed by drug delivery of piperine 20% only in the right leg. Blind evaluators conducted the assessment through photographs and comparative measurements of the lesions.

Results: The lesion size ranged from 0.2cm to 1.5 cm. Both treatments significantly reduced lesion size ($p< 0.00008$, right leg; $p< 0.002$, left leg). The generalized estimating equation method showed that there was no statistical difference between the groups regarding the reduction of lesion size. The evaluation achieved better scores for the right leg (laser + piperine) ($p=0.126$).

Conclusion: Although statistically significant differences were not found between the side treated with laser and drug delivery versus the side treated only with laser, we believe that the technique may represent an option in the therapeutic arsenal.

Keywords: Administration, topical; Drug administration routes; Lasers

RESUMO

Introdução: *Introdução: A hipomelanose gutata idiopática constitui uma leucodermia adquirida, cujas opções terapêuticas disponíveis apresentam resultados inconsistentes.*

Objetivos: *Avaliar o efeito do laser fracionado ablativo isolado ou combinado ao drug delivery de piperina para tratamento de hipomelanose gutata idiopática.*

Materiais e métodos: *Em um estudo prospectivo e comparativo, sete pacientes apresentando HGI nas pernas foram selecionadas, sendo escolhidas cinco lesões em cada perna de cada uma. Ambas as pernas de cada paciente foram submetidas a duas sessões de laser Er:YAG 2940nm, seguido de drug delivery de piperina 20% somente na perna direita. A avaliação foi feita por avaliadores cegos por meio de fotografias e medidas comparativas das lesões.*

Resultados: *O tamanho das lesões variou de 0,2cm a 1,5cm. Ambos os tratamentos reduziram significativamente o tamanho das lesões ($p<0,00008$ perna direita e $p<0,002$ perna esquerda). O método de equações de estimativas generalizadas demonstrou que não houve diferença estatística entre os grupos em relação à redução do tamanho das lesões. A avaliação atingiu melhores escores para a perna direita (laser + piperina) ($p=0,126$).*

Conclusão: *Embora diferenças estatisticamente significativas não tenham sido encontradas entre o lado tratado com laser e drug delivery versus o lado tratado somente com laser, acreditamos que a técnica possa representar uma opção no arsenal terapêutico.*

Palavras-Chave: *Lasers; Vias de administração de medicamentos; Administração tópica*

INTRODUCTION

Idiopathic guttate hypomelanosis (IGH) is an acquired leukoderma that begins to appear in middle-aged individuals.[1] IGH represents an event of the skin aging process, along with senile lentigos and whitening of the hair, among others.[2] Clinically, IGH presents as hypopigmented, asymptomatic, rounded or oval macules in photoexposed areas, mainly in limbs.[1] The pathogenesis of IGH is not completely elucidated: senile degeneration, exposure to ultraviolet radiation, trauma, autoimmunity, and local inhibition of melanogenesis may be involved.[3,4] Also, lesions, in general, are accompanied by other photodamage signals, and some authors believe that its pathogenesis may be similar to that observed in hair whitening.[2]

Therapeutic options for IGH treatment include topical tretinoin, calcineurin inhibitors, cryotherapy, dermabrasion, and laser fractional photothermolysis; however, treatment results are sometimes discouraging in clinical practice.[5-10] The use of ablative fractional laser is described in the literature; in one study, the CO2 laser 10,600 nm achieved improvement over 75% in 47.9% of patients two months after treatment with a single session; another study compared the use of CO2 laser 10600 nm with Er:YAG 2940 nm *versus* 0.025% topical tretinoin and observed better results in the group receiving the laser treatment ($p<0.01$). [1,10]

Melanocytes are dendritic cells that produce melanin, the main component responsible for cutaneous pigmentation.[2] They are located mainly in the skin, more specifically in the basal layer of the epidermis and in the matrix of the hair follicles, also found in the iris, the cochlea, and the midbrain.[2] The melanin production occurs in cellular organelles called melanosomes and, later, melanin is transferred to the epidermal keratinocytes.[2]

Piperine, the main alkaloid present in black pepper (*Piper nigrum L., Piperaceae*), has stimulatory effects on the replication and dendrite formation of melanocytes, both *in vitro* and *in vivo*.[11,12] The mechanism suggested in this melanocytic stimulus appears to be mediated by the protein kinase C (PKC) since this stimulus can be counteracted by PKC inhibitors.[11] Also, piperine does not bind to DNA when applied to the skin, such as occurs with the use of psoralens, so they do not form photoadducts.[13]

The drug delivery assisted by ablative fractional lasers allows to optimize the skin permeation of drugs by tissue ablation, which favors its use for several topical medications.[14] To date, drug delivery assisted by ablative fractional lasers has not been reported in the literature as an option for IGH treatment; for this reason, the authors conducted this pilot study in order to evaluate the effect of piperine drug delivery.

OBJECTIVES

To assess the effect of isolated or combined ablative fractional laser on the immediate topical application of piperine (drug delivery) for the treatment of IGH by means of a pilot, prospective, and comparative study.

MATERIALS AND METHODS

Seven female patients, aged 55-66 years, presenting IGH in the legs, were selected for this pilot study. All patients signed an informed consent form, and the study was conducted according to the rules of the Declaration of Helsinki. Five lesions were selected on each leg of each patient, which were measured for their largest diameter to allow objective comparative analysis at the end of the study. Both legs of each patient were submitted to two monthly sessions of ablative fractional laser Er:YAG 2940 nm (Etherea MX®, Vydence Medical, São Carlos, São Paulo, Brazil) 8mm, 100 MTZ/cm^2, single mode (500µs) with an energy of 12.5mJ/MTZ, followed by cleaning with 0.9% saline immediately after the procedure to remove debris. Soon after the end of the described laser protocol, an anhydrous serum formulation containing 20% piperine was applied (drug delivery) only in the right leg of each patient; the patients were instructed to apply the formulation for three days after the procedure, three times a day. An important consideration is that piperine undergoes photoisomerization and loses its ability to stimulate melanocytes if exposed to visible light or ultraviolet radiation; for this reason, patients were instructed to avoid sun exposure during the application period of the product.[13] The patients were evaluated one month after the second session; on this occasion, all lesions were measured again at their largest diameter and photographic records of the lesions were performed for the subjective analysis of the results.

Subjective evaluation

The subjective evaluation of IGHs was conducted by two dermatologists blinded to the type of treatment performed in each leg. The following score was used to evaluate the improvement: 1 - worsening; 2 - no response; 3 - mild improvement (reduction of lesion size between 25-50%); 4 - good response (reduction of lesion size between 50-75%); 5- excellent response (reduction of lesion size over 75%).

Statistical analysis

Data were analyzed by SPSS version 21.0 (IBM Corporation, United States). Quantitative variables were compared between groups (laser + piperine *versus* laser alone) before and after treatment using the generalized estimating equation (GEE) method and the Bonferroni test. The paired T-test was used to evaluate the response to treatment (before and after) of each leg.

RESULTS

Seven patients were selected; six were submitted to two sessions and one to only one session (Table 1).

In total, 35 IGH lesions were treated in the right legs of the patients and 35 in the left legs. The size of the lesions ranged from 0.2cm to 1.5cm. The mean pre-treatment lesion size was 0.58cm in the right leg, where piperine was applied in drug delivery (confidence interval [CI]: 0.45-0.7 cm; standard deviation [SD]: 0.06cm) and 0.59 cm in the left leg (CI: 0.49-0.68cm; SD: 0.05cm), where only the laser was performed. Thirty days after the second session, the mean lesion size in the right leg

was 0.39cm (CI: 0.28-0.51cm; SD: 0.06cm) and 0.45cm in the left leg (CI: 0.34-0.55cm; SD: 0.05cm) (Table 2). The paired T-test demonstrated that both treatments significantly reduced the lesion size ($p<0.00008$ for the right leg and $p<0.002$ for the left leg). The GEE method showed that there was no difference between the groups regarding the reduction of the size of the lesions (Bonferroni test: 0.083). The subjective evaluation reached better scores for the right leg (laser + piperine) (mean 4.72 *versus* 4.28 for the left leg); however, this difference did not reach statistical significance ($p=0.126$).

The photographic evaluation record of the clinical result achieved can be observed in Figure 1.

Table 1 : Sample Characteristics

	n=7
Age - years (M±SD)	55-66 (61.83±4.07)
Sex - Female (n; %)	7; 100
Phototype*	**(n; %)**
II	3; 42
III	4; 58
Number of sessions performed	**(n; %)**
1	6; 86
2	1; 14
IGH size before treatment	
Right leg (M±SD)	0.58cm ± 0.26cm
Left leg (M±SD)	0.39cm ± 0.20cm

M = mean, SD: standard deviation

Table 2: Mean lesion size reduction 30 days after the second treatment session on the right and left legs

	Right leg (n=35)	Left leg (n=35)	P-value
Mean reduction in lesion diameter after treatment	0.18cm (SD 0.14cm)	0.14cm (SD 0.15cm)	0.21

SD: standard deviation * P-value <0,05

DISCUSSION

The ablative fractional laser produce ablation microchannels, surrounded by a thin coagulation zone, which facilitates the permeation of topical medications applied to the skin immediately after the procedure, this process is called laser-assisted drug delivery. A positive aspect of this method is the fact that the laser technique allows modulating the microchannels by changing the parameters used in the laser device. Thus, it is possible to modulate them in terms of size, depth and distance between each other.[15] A noteworthy fact is that the formed microchannels are more permeable in the first hours after the procedure and, therefore, the medication should be applied immediately after the laser, as it was performed in this pilot study.[15]

When we think about the IGH treatment, it is important to try to understand its pathogenesis: there seems to be an associated genetic component that favors the physiological aging of melanocytes,[16] as well as physical, chemical and biological factors that can cause DNA mutations resulting in depletion of stem cells in melanocytes.[17] Also, TGF-beta signaling appears to be involved in the process.[18]

Some authors believe that there is an active process of depigmentation by inhibition of melanogenesis in IGH lesions. This hypothesis justifies the use of piperine as a therapeutic option.[19] Some studies have evaluated the use of piperine for vitiligo treatment. A clinical study that assessed 18 vitiligo lesions treated in three patients observed repigmentation in the lesions with the use of piperine. Both the use of the *Piper nigrum* extract and that of the purified piperine in ointment vehicle promoted the pigmentation.[20] The same study demonstrated the antioxidant activity of piperine by spectrophotometry.[20] The use of piperine associated with narrow-band UVB phototherapy was described in a clinical study that evaluated 63 patients with facial vitiligo and observed superiority in repigmentation in patients in the group that received the application of piperine associated with phototherapy in relation to those who only performed isolated phototherapy ($p<0.0001$).[21] In this case, the 1% piperine solution was applied one hour after the phototherapy since it is

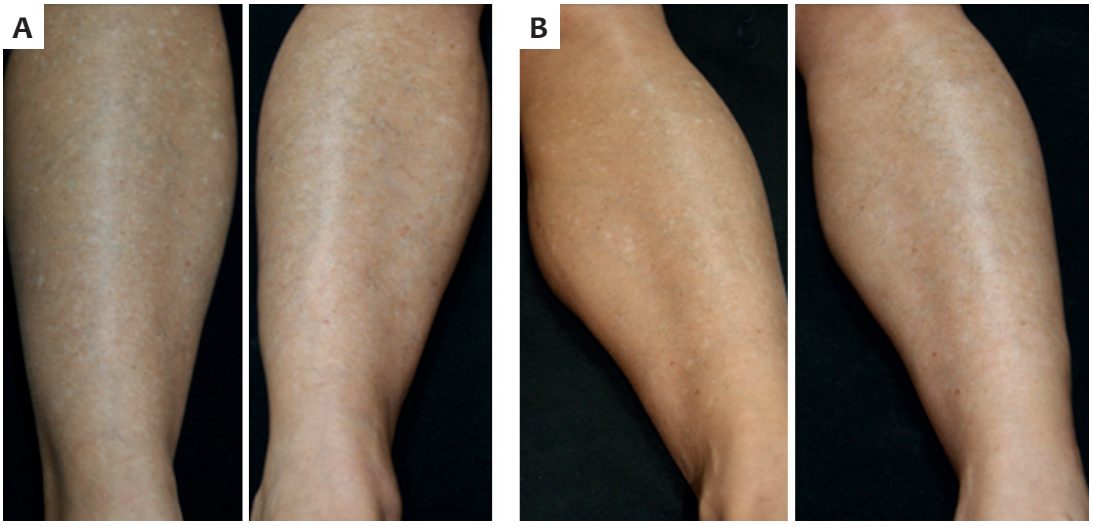

Figure 1: Patient 1-A: right leg before and after Laser Er:YAG 2940nm and piperine drug delivery; **B:** left leg before and after pictures Laser Er:YAG 2940nm.

photoisomerized and inactivated if it is applied before the phototherapy session. Adverse events described with the application of piperine include burning sensation and erythema.[21] To date, no study has evaluated the effect of piperine on the treatment of IGH.

Choosing the right vehicle for drug delivery is also extremely important. In the present study, we chose the vehicle anhydrous fluid serum, which has low viscosity and allows occlusion, and these factors potentiate the permeation of the assets added to it. Also, the vehicle is free of preservatives that could cause contact dermatitis or foreign body reaction.

Although our study found no statistically significant differences between the side treated with laser associated with piperine drug delivery and the side treated with ablative fractional laser alone, we believe that the technique may represent an option in the therapeutic arsenal of IGH. Limitations of the study include the small sample size, which was possibly one of the factors involved in the fact that no statistically significant differences were observed between treatment outcomes, despite the positive trend of higher response on the side receiving the associated drug delivery. Even if a result is not statistically positive due to the small sample size, in this case, the variability between the groups tends to have a significant impact. The mean of one group was higher than that of the other and, possibly, a larger group could demonstrate the superiority of the proposed technique. The small number of sessions and the short follow-up time are also relevant limitations. Since this is a pilot study, we assume that future studies, with larger sample size and a longer follow-up period, may clarify the doubts that have remained.

CONCLUSIONS

The search for effective treatments for IGH has not yet reached its final goal. The results of the currently available treatments are variable and often unsatisfactory. Such dissatisfaction with the results occurs both on the part of the dermatologist and the patient. The optimization of drug administration routes through drug delivery, as presented in this study with piperine, has been discussed for a number of dermatological conditions. Although the results of this pilot study are not definitive, we provide an option to be remembered when other therapies have not been effective in the treatment of IGH.

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Pre-clinical evaluation of the profilatic effects of Pinus pinaster extract (Pycnogenol®) on skin hemosiderin deposits

Avaliação pré-clínica dos efeitos profiláticos do extrato de Pinus pinaster (Pycnogenol®) sobre a deposição cutânea de hemossiderina

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ABSTRACT

Introduction: *Sclerotherapy is the most widely used method for the treatment of varicose veins of the lower limbs. The most common complication is the appearance of hyperchromic spots in the treated region. Pycnogenol® has long been known as a phlebotonic, anti-inflammatory and skin depigmenting drug. Studies have already proven the efficacy of this drug in the prevention and treatment of post inflammatory hyperpigmentation.*

Objective: *To evaluate the effectiveness of Pinus pinaster extract (Pycnogenol®; PPE) in the prevention of hemosiderin deposits in human skin culture submitted to inflammatory stress.*

Methods: Fragments of human skin were stimulated with interleukin 1 alpha (IL-1 a*) to induce an inflammatory response and, concurrently, treated with PPE for further histological evaluation and hemosiderin semi-quantification.*

Results: *The histological evaluation of skin fragments exposed to IL-1 alpha revealed a 26.6% higher hemosiderin density compared with the control group. Moreover, skin fragments incubated concomitantly with PPE showed significant reductions in hemosiderin deposits when compared with the group only exposed to the inflammatory microenvironment.*

Conclusions: *The results presented in this study showed an important effect of PPE (Pycnogenol®) in the prevention of hemosiderin accumulation caused by inflammatory stress similar to the post-sclerotherapy process.*

Keywords: *Hemosiderin; Hyperpigmentation; Sclerotherapy*

RESUMO

Introdução: A escleroterapia é o método mais utilizado para o tratamento de varizes dos membros inferiores tendo como complicação mais comum o aparecimento de manchas hipercrômicas na região tratada. O Pycnogenol® é conhecido há muito tempo como um flebotônico, anti-inflamatório e despigmentante da pele. Estudos já comprovaram a eficácia deste fármaco na prevenção e no tratamento da hiperpigmentação pós-inflamatória.

Objetivo: Avaliar a eficácia do extrato de Pinus pinaster (Pycnogenol®; EPP) na prevenção de depósitos de hemossiderina em cultura de pele humana submetida a estresse inflamatório.

Métodos: Fragmentos de pele humana foram estimulados com interleucina 1 alfa (IL-1a) para indução de uma resposta inflamatória e, concomitantemente, tratados com EPP para posterior avaliação histológica e semi-quantificação de hemossiderina.

Resultados: A avaliação histológica dos fragmentos de pele expostos à IL-1alfa; revelaram uma densidade de hemossiderina 26,6% maior em comparação ao grupo controle. Por outro lado, os fragmentos de pele incubados concomitantemente com EPP mostraram reduções significativas na deposição de hemossiderina quando comparados ao grupo somente expostos ao microambiente inflamatório.

Conclusões: Os resultados apresentados neste estudo apontam para um importante efeito do EPP (Pycnogenol®) na prevenção do acúmulo de hemossiderina originado pelo estresse inflamatório semelhante ao processo pós escleroterapia.

Palavras-chave: Hemossiderina; Hiperpigmentação; Escleroterapia

Original Articles

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INTRODUCTION

Chronic venous insufficiency (CVI) is the most prevalent of venous diseases, with national data showing rates ranging from 35% to 50% of the population.[1] In the twentieth century, several theories were proposed regarding the etiology and pathophysiology of CVI, with more discussion regarding hypertension secondary to reflux and/or obstruction in the venous system. These abnormalities may present features ranging from telangiectasias to more severe conditions such as phlebitis and ulcers (CEAP C0 to C6).[2]

Sclerotherapy is the most widely performed treatment in the world for varicose veins in all its stages. Knowledge of the technique and the adverse events of each substance can optimize the results. However, each treatment presents a series of complications, especially when using microfoam for the treatment of larger caliber veins.[3]

The development of post-sclerotherapy hyperchromic spots may occur due to two main factors: hemosiderin deposition and post-inflammatory pigmentation. Hemosiderin is a pigment found in the dermis that results from the extravasation of red blood cells from treated vessels and also from the inflammatory reaction generated by the excessive presence of iron and the formation of free radicals. This pigment presents an ocher color of difficult removal.[4-5] Post-inflammatory hyperpigmentation occurs due to the melanocytic reaction to the presence of metallic ions, such as iron and inflammatory kinins, such as interleukin 1 (IL-1), IL-6, and nuclear transcription factor kappa B (NFkB), especially in patients with higher phototype or melasma.[6]

Most of these spots progress to spontaneous bleaching in up to six months; however, about 2% of these spots remain for more than a year. The early treatment can favor resolution and prevent its chronification, which generates great anxiety to the patient, who will blame the doctor for the damage caused.[7]

Pycnogenol® (FQM Famoquímica S/A, Rio de Janeiro, Brazil) is a standardized extract of the bark of the French maritime pine (Pinus pinaster), rich in procyanidins. This substance has proven antioxidant and anti-inflammatory actions, acting in the reduction of lower limb edema, improvement of symptoms related to CVI, reduction of melanogenesis and, consequently, reduction of post-inflammatory hyperpigmentation.[8-9]

This study aimed to evaluate the efficacy of a Pinus pinaster (Pycnogenol®) extract in the prevention of hemosiderin deposits in human skin culture submitted to inflammatory stress.

METHODS

The dry Pinus pinaster extract (PPE) was supplied by the FQM Farmoquímica S/A, Rio de Janeiro, Brazil (Flebon® 50 mg), which contains: Pycnogenol®, corn starch, alpha-cellulose, silica (silicon dioxide), magnesium stearate, sodium carboxymethylcellulose, sodium lauryl sulfate and sodium carboxymethyl starch.

This study used fragments of human skin from a healthy woman, phototype II, 54 years old, who underwent elective plastic surgery in the abdominal region (abdominoplasty). After the surgical procedure, the skin fragments were collected in plastic bottles containing 0.9% saline and kept in refrigeration for up to 24 hours. The fragments were fractionated into approximately 2 cm^2 pieces, then weighed, incubated in a 24-well plate (Nunc, Denmark) with DMEM (Dulbecco's Modified Eagle's Medium; Corning, USA) containing glucose, L-glutamine, penicillin, streptomycin and fetal bovine serum (Thermo Fisher Scientific, USA).

The PPE concentrations assessed in this study were 0.100, 0.0316, and 0.0100 mg/mL, previously determined by the cell viability assay (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide). For induction of an inflammatory environment, the cultures were concomitantly stimulated with interleukin-1 alpha (IL-1α; 10 ng/mL; BioLegend, USA) for a period of 48 hours.

After the treatment and application of inflammatory stress, the skin fragments were fixed in paraformaldehyde for 24 hours and cryoprotected in sucrose solution for 72 hours. Then, serial cuts of 10 µm were collected directly on silanized slides with the aid of Cryostat (Leica Microsystems, Germany) and stained with Perls' Prussian Blue technique.[10] For better visualization of the cuts, a counterstaining was performed using an aqueous solution containing 0.1% safranin and 1% glacial acetic acid. The presence of hemosiderin was evaluated under an optical microscope (BX53, Olympus Corporation, Shinjuku, Tokyo, Japan) using the cellSens Standard software (© 2010, Olympus Corporation). After obtaining the images, hemosiderin was semi-quantified (arbitrary units - AU) with ImageJ software (version 1.49V, National Institutes of Health, Bethesda, MD, USA). The images were divided into blue, red and green channels, and blue channel images were binarized for pixel quantification.

The use of fragments of human skin in elective surgeries for this study was approved by the Committee for Ethics in Research of the São Francisco University (SP).

Statistical analysis was performed by ANOVA (GraphPad Prism v6) to measure the variation of the results, comparing the data among all groups. We also applied the Bonferroni post-hoc test, which strengthened and made the results more accurate. Statistical significance was considered as $P<0.05$. Data were expressed as mean ± standard deviation.

RESULTS

Figure 1 represents the cell viability curve for PPE. The product had non-cytotoxic concentrations from 0.100 mg/mL.

Figure 2 represents the histological evaluation of hemosiderin by the Perls' Prussian Blue technique on fragments of human skin obtained from the elective plastic surgery, incubated with PPE and submitted to IL-1α inflammatory stress. Figure 3 shows the semi-quantification of this pigment. As we can observe, fragments of skin exposed to IL-1α have 26.6% greater hemosiderin density compared with the control group ($P<0.001$, Figure 3). However, skin fragments incubated with PPE and exposed to the inflammatory microenvironment show significant reductions in hemosiderin deposits when compared with the only group exposed to IL-1α. The observed reductions

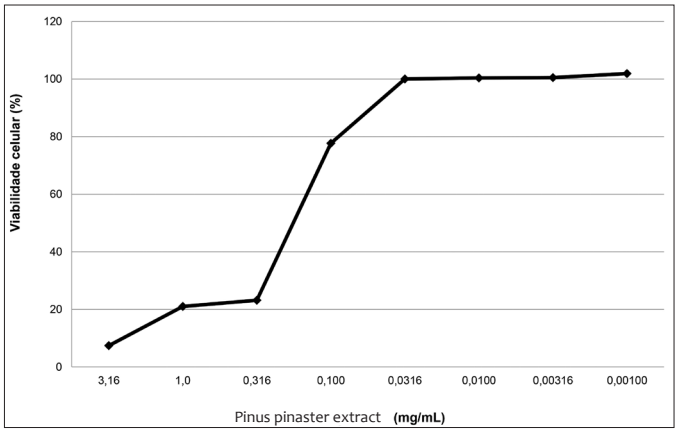

Figure 1: Evaluation of non-cytotoxic concentrations of Pinus pinaster extract (EPP) in human fibroblast culture after 48 hours of incubation by the MTT assay

were 24.55; 26.39 and 25.93%, respectively, at concentrations of 0.100; 0.0316; and 0.0100 mg/mL of PPE (P<0.001; Figure 3).

DISCUSSION

The choice of the therapeutic method for the treatment of varicose veins should be made based on the degree of affection and the characteristics of the patient, such as skin phototype and the use of concomitant medications, which may predispose or aggravate skin spots. However, once the hyperchromia is installed, the evolution time must be respected, because most of the time a spontaneous resolution occurs within six months. [7]

When the outcome is unfavorable, it is common to observe a scenario with some degree of fragility and anguish, since the presence of post-sclerotherapy spots increases the degree of aesthetic expectations and emotional factors. Therefore, during the therapeutic approach to hyperchromia, it is of utmost importance to establish a longitudinal connection with the patient, who will often present a pressing need for outcome. To discuss the duration, indications, and limitations of each proposed method brings clarity and confidence, with greater adherence to the treatment and possibility of satisfactory results. [7]

During the sclerotherapy, an endothelial lesion may occur with exposure of subendothelial collagen fibers, causing platelet aggregation and chemotactic factors release. Consequently, vessel thrombosis occurs and the clot formed leads to fibroblast proliferation and fibrotic organization. Cutaneous hematomas originate from the extravasation of blood into the surrounding connective tissue with activation of the inflammatory response and migration of specialized cells to the affected site. The area is first infiltrated with neutrophilic granulocytes and then with macrophages, the latter being responsible for phagocytosis of erythrocyte residues, particularly hemosiderin, which are detected by the specific Perls' Prussian Blue method. [10]

Preliminary studies conducted by our group demonstrated the effects of Pycnogenol® on the prevention of melanogenesis using fragments of human skin exposed to ultraviolet radiation A and B, infrared radiation A, and visible light, corrob-

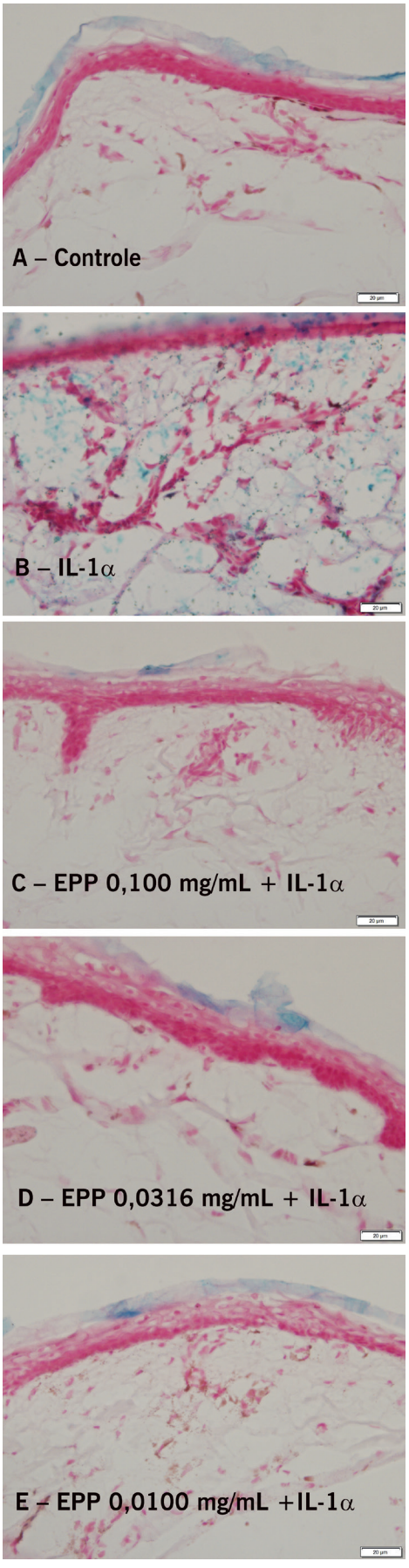

Figure 2: Histological evaluation of hemosiderin by the Perls' Prussian Blue staining in ex vivo skin fragments treated with Pinus pinaster extract (PPE) and submitted to inflammatory stress with interleukin 1α (IL-1α). A: control group; B: inflammatory stress with IL-1α; C: PPE 0.100 mg/ mL + IL-1α; D: PPE 0.0316 mg/ mL + IL-1α; E: PPE 0.0100 mg/ mL + IL-1α. The hemosiderin pigments are marked in blue. The images were obtained with a 40x objective lens and the reference bar corresponds to 20 µm

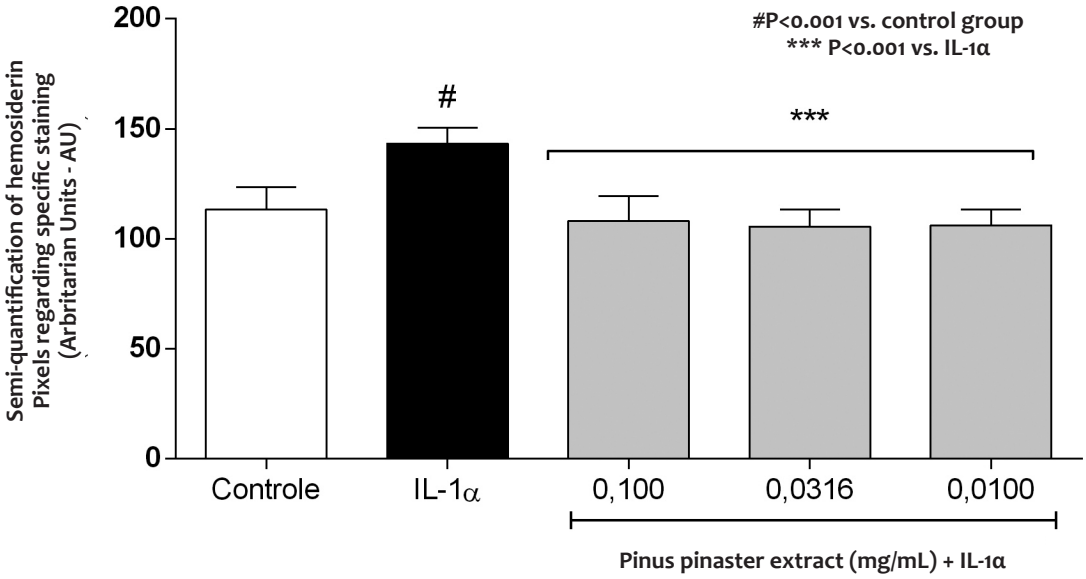

Figure 3: Semi-quantification of hemosiderin obtained by the Perls' Prussian Blue staining in ex vivo skin fragments treated with Pinus pinaster extract (PPE) and submitted to an inflammatory microenvironment with interleukin 1α (IL-1α). Data represent the mean ± standard deviation of 10 experimental areas (Anova, Bonferroni)

orating the bleaching benefit after sun exposure.[11] Similar results were observed by Kim (2008), who pointed out that Pycnogenol® has an antimelanogenic effect, inhibiting the activity of the tyrosinase enzyme involved in the production of melanin by mechanisms that encompass free radical suppression and positive regulation in B16 cells.[12]

Pycnogenol® and its action on microcirculation were exalted by Fitzpatrick *et al.* (1998) and later by Belcaro *et al.* (2016). Both authors have demonstrated that the extract causes a significant prevalence of oxygen in the dermis, also reducing the presence of carbon dioxide. They also emphasized the stimulating effect of endothelial function resulting from the prolonged synthesis of endothelial nitric oxide.[13-14]

Bascones *et al.* (2006) observed that the treatment of human fibroblasts with 0.100 mg/mL of PPE resulted in the inhibition of damage caused by the oxidative stress. In addition, this extract demonstrated a regulation of the expression of matrix metalloproteinases and the synthesis of pro-collagen type I by inhibition of activating protein 1 (AP-1), a transcription factor that responds to inflammatory stimuli.[15]

The present study was conducted aiming to assess the effects of the dry Pinus pinaster extract (PPE) on the hemosiderin deposits using an *ex vivo* model of human skin culture. Histological parameters were investigated in skin explants treated with PPE and stimulated concomitantly with interleukin 1 alpha (IL-1α) for the induction of inflammatory microenvironment.

Our results demonstrated a significant increase in hemosiderin staining in the group exposed only to IL-1α. However, the skin fragments treated with PPE presented hemosiderin staining similar to that observed in the basal control group. Our results are in agreement with data from the literature that report antioxidant and anti-inflammatory actions of the Pinus pinaster extract in *in vitro* research models.[15]

To date, no study has demonstrated the activity of Pinus pinaster extract in the cutaneous hemosiderin deposits. Thus, we provide original data on the efficacy of PPE in the prevention of hemosiderin deposits in cultured human skin submitted to inflammatory stress, similar to the environment created after sclerotherapy.

CONCLUSIONS

The results presented in this study point to an important effect of the Pinus pinaster extract (Pycnogenol®) in the prevention of hemosiderin accumulation originated by inflammatory stress similar to the post-sclerotherapy process. Although additional studies are required to prove the prophylactic activity in local hyperpigmentation, the use of the Pinus pinaster extract (Pycnogenol®) can be considered as a promising option, differentiating it from the other substances currently available.

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Histological profile of melanocytic lesions excised in a Dermatology service, based on clinical and dermoscopic criteria

Perfil histológico das lesões melanocíticas excisadas em um serviço de Dermatologia, com base em critérios clínicos e dermatoscópicos

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ABSTRACT

Introduction: The gold standard diagnosis of cutaneous melanoma is essentially histological. Dermoscopy adds to clinical examination improved sensitivity and specificity.
Objective: To evaluate the histological profile of risk melanocytic lesions excised in a dermatology service.
Methods: Case review through Excel spreadsheet and survey of dermoscopic, histological and topographic data (January 2017 to December 2018).
Results: Data from 62 suspected melanoma lesions were evaluated in this period. The main histological results were: melanocytic nevi (37.1%), melanoma (19.35%), and dysplastic nevi (17.7%).
Conclusions: The positive predictive value for melanoma was 19.35%, considering the search for an early diagnosis in patients at risk.
Keywords: Neoplasms; Melanoma; Dermatology

RESUMO

Introdução: *O diagnóstico padrão-ouro do melanoma cutâneo é essencialmente histológico. A dermatoscopia agrega ao exame clínico melhora da sensibilidade e especificidade.*
Objetivo: *Avaliar o perfil histológico das lesões melanocíticas de risco excisadas em um serviço de dermatologia.*
Métodos: *Revisão dos casos por meio da tabela Excel e levantamento de dados dermatoscópicos, histológicos e topográficos (janeiro de 2017 a dezembro de 2018).*
Resultados: *Foram avaliados neste período dados de 62 lesões suspeitas de melanoma. Os principais resultados histológicos foram: nevos melanocíticos (37,1%), melanoma (19,35%) e nevos displásicos (17,7%).*
Conclusões: *O valor preditivo positivo para melanoma foi 19,35%, considerando a busca por um diagnóstico precoce em pacientes de risco.*
Palavras-chave: *Neoplasias; Dermoscopia; Melanoma*

INTRODUCTION

Melanoma is a neoplasm with a high probability of metastasis. Early diagnosis is a function of the dermatologist since this significantly improves the patient's prognosis.[1] Patients with multiple nevi, dysplastic nevus syndrome, personal or family history of melanoma, and with spots in transformation above the age of 35 should be considered at risk.

The gold standard diagnosis of cutaneous melanoma is essentially histological.[2] The clinical aspects of melanocytic lesions (nevi) contribute to the clinical hypothesis. The ABCDE rule is used (A- Asymmetry; B- border irregularity; C- color variation; D- Diameter >6 mm; E-Evolving, which includes rapid growth, pruritus, bleeding, and ulceration). The greater number of these signs indicates a higher probability of melanoma.[1]

Dermoscopy (polarized light that allows visualization of epidermal and dermal structures) is a feature that increases the accuracy of clinical examination and allows the differential diagnosis between a melanocytic and a non-melanocytic lesion.[2] Among melanocytic lesions, the pattern analysis stratifies its risk category but its definitive diagnosis will be histological. Through the clues to malignancy, the dermoscopy also contributes to the excision indication of initial atypical melanocytic lesions and melanomas *in situ*. In addition, comparative analysis of multiple nevi patterns in the same patient may define the excision of a chaotic lesion with a different pattern ("ugly duckling sign").[3] This method provides sensitivity ranging from 62% to 94 %.[2] Procianoy, 2009, also demonstrated the sensitivity of the dermoscopy through the Pattern Analysis method, finding the value of 91.7%, while the specificity of the method was 41.7%.[2] It has already been shown that dermoscopy increases sensitivity and specificity of the melanoma diagnosis in 35% when compared with the clinical diagnosis.[2] It is important to remember that dermoscopy has been contributing to an earlier diagnosis of melanoma. Currently, in Sweden, 40% of the diagnosed melanomas are *in situ* (in 1996 this number was 20%).[4]

There are several methodologies based on dermoscopy to stratify the risk of melanocytic lesions. The "Chaos and Clues" (Kitler H) method is a simple algorithm to facilitate the medical decision as to whether or not to excise a certain lesion.[3] For lesions exhibiting chaos (asymmetry of structures and/or color), one must look for clues to malignancy [3] (Table 1 of clues to malignancy for pigmented lesions exhibiting chaos). This method showed a sensitivity of 90.6% and specificity of 62.7% (malignancies) in a study involving 463 excised pigmented lesions, 29 of which were melanomas.[3]

Analyzing the dermoscopy, sensitivity is the method ability to detect melanoma in the population. Usually, these numbers are expressive among the several algorithms, i.e., although many benign lesions are removed, melanomas are very little unnoticed. Moreover, specificity is the ability to find benign lesions in the population. This number is expressive but tends to be smaller than the sensitivity because there are many cases of false positive (considering that dysplastic nevus or even melanocytic nevus may present chaos to dermoscopy and/or evolve with growth asymmetry). The positive predictive value regarding an examination is: "since this examination was abnormal, the probability is that the individual is actually sick."[5] For the same reason of the specificity, its value tends to be smaller than that of the sensitivity and even smaller than that of the specificity because, in the universe of the melanocytic lesions, finding "normal" is a simpler task than trying to diagnose an initial melanoma considering that in this evolutionary process nevi, dysplastic nevi and melanomas may present the same dermoscopic characteristics without a specific feature to differentiate them, revealing at this moment the dermoscopy limitation despite its unquestionable importance.

OBJECTIVES

To evaluate the histological profile of excised melanocytic lesions at risk, based on clinical standards, clinical history, and dermoscopic method.

METHODS

A study conducted at the Dermatology Service of the Universidade de Mogi das Cruzes, São Paulo, Brazil, from January 2017 to December 2018, which assessed 62 suspected melanoma lesions.

Retrospective, epidemiological study analyzing the information contained in the Excel spreadsheet of suspicious melanocytic lesions with clinical, dermoscopic, and histological findings. Analysis of medical records of the UMC Polyclinic. The research was approved by the Committee for Ethics in Research of the UMC; CAAE (Presentation Certificate for Ethical Appraisal) number 60657516.0.0000.5497. Data from adults with melanocytic lesions at risk from UMC were assessed during 2017 and 2018. Clinical description and dermoscopy were the criteria used to choose excision. In this period several dermoscopic algorithms were used, but mainly Chaos and Clues (year 2018), so we tried to standardize this study by adapting the other dermoscopic descriptions to the elements of this one that was the most practiced method. The evaluation of the results was conducted from the correlation with the histological reports.

The lesions were recorded in a database with the following information: diagnostic hypothesis, age, sex, and lesion topography. After this step, the diagnostic hypothesis (clinical and dermoscopic data) was compared with the final histological result.

RESULTS

Sixty-two skin lesions with a diagnostic hypothesis of melanoma were assessed, being 24 men (38.7%) and 38 women (61.3%), with a mean age of 56 years (Table 1). The body loca-

Chart 1: Dermoscopic clues to malignancy

Dermoscopic signs to malignancy
1 Thickened reticular lines
2 Asymmetrically distributed black spots and lumps (on the periphery)
3 Radial lines; pseudopods (segmental)
4 Sites without structure (eccentric)
5 Blue or gray structures
6 White radial lines
7 Polymorphous vessels
8 Polygons (angled lines)
9 Pigment (parallel lines) in the epithelial ridge (palmoplantar melanoma)
10 Irregular pigment band (nail melanoma)

tion with the most biopsied lesions was the trunk with 30.6% of the cases as shown in Table 2.

Dermoscopy showed a greater proportion of lesions with spots and lumps asymmetrically distributed in 25.8% of the cases, followed by areas without structure (16.1%) according to Table 3.

As for the histological results (definitive diagnoses), melanocytic nevi were the most frequent lesions (37.1%), followed by melanomas with 19.4% of the cases (Table 4).

Of the confirmed cases of melanoma, the order of frequency of the finding 2 (Chart 1) coincided with the general excision, i.e., it was the most found, followed by the radial lines/pseudopods (two cases) (Table 5).

Table 1 : Gender

	Frequency	Percentage	Valid percentage
Men	38	61.3	61.3
Women	24	38.7	38.7
Total	62	100.0	100.0

Table 2 : Great area location

	Frequency	Percentage	Valid percentage
Chest and back	19	30.6	30.6
Head and neck	12	19.4	19.4
Lower limbs	10	16.1	16.1
Palmoplantar	11	17.7	17.7
Upper limbs	8	12.9	12.9
Not informed	2	3.2	3.2
Total	62	100.0	100.1

Table 3 : Dermoscopic findings

	Frequency	Percentage	Valid percentage
"2"	16	25.8	25.8
"4"	10	16.1	16.1
"1"	5	8.1	8.1
2 and 4	5	8.1	8.1
No dermoscopic description	4	6.5	6.5
"3"	3	4.8	4.8
"8"	3	4.8	4.8
"5"	2	3.2	3.2
"7"	2	3.2	3.2
2 and 5	2	3.2	3.2
"10"	1	1.6	1.6
"9"	1	1.6	1.6
2, 4 and 7	1	1.6	1.6
2, 4 and 8	1	1.6	1.6
3 and 4	1	1.6	1.6
3 and 5	1	1.6	1.6
3 and 7	1	1.6	1.6
4 and 5	1	1.6	1.6
5	1	1.6	1.6
5 and 8	1	1.6	1.6
Total	62	100.0	100.0

DISCUSSION

Dermoscopy is an instrument of great importance for the screening of risk diagnoses: low, moderate, and high – criteria

Table 4 : Histological results

	Frequency	Percentage	Valid percentage
Melanocytic Nevus	23	37.1	37.1
Melanoma	12	19.4	19.4
Dysplastic nevus	11	17.7	17.7
Other	10	16.1	16.1
Blue nevus	4	6.5	6.5
Reed nevus	2	3.2	3.2
Total	62	100.0	100.0

Table 5 : Clues to Malignancy X Melanoma

Clues to Malignancy	N of occurrences
"2"	3
"3"	2
"10"	1
"4"	1
"8"	1
2 and 4	1
3 and 7	1
"5"	1
No dermoscopic description	1
"1"	0
"5"	0
"7"	0
"9"	0
2, 4 and 7	0
2, 4 and 8	0
2 and 5	0
3 and 4	0
3 and 5	0
4 and 5	0
5 and 8	0
Total	12

Calculation of positive predictive value (PPV)
PPV = True positives / True positives + False positives
PPV = 12/12 + 50
PPV = 12/62
PPV = 19.35

that can be used for melanocytic and non-melanocytic lesions. The knowledge of the patterns that allow such stratification defines an appropriate behavior before the patient.

The present study assessed patients with supposedly melanocytic lesions (clinically suspected melanoma or nevoid), with moderate or high risk for melanoma. The following were considered in the decision for excision: personal or family history of melanoma, clinical history, clinical aspect (ABCDE), and multiple nevi, defining the patient at risk.

The dermoscopic "Chaos and Clues" pattern contributes to the more invasive decision in dubious cases. The higher number of patterns (dermoscopic chaos) indicated a greater probability of melanoma. However, a single pattern among clues to malignancy (clue to malignancy Table) may already represent a melanoma (usually initial: the diagnostic precocity is a major goal in patients at risk or not necessarily at risk). This is what is observed in Table 5: leading cases of melanoma with only one pattern (which is a clue to malignancy): spots and lumps asymmetrically distributed.

A large number of excised benign lesions (melanocytic nevus) is evident, demonstrating that despite the sum of data that confirms the risk, they do not define the diagnosis, which is essentially histological. Carrera *et al.*, when comparing dermoscopic teaching algorithms, found modest levels of diagnostic accuracy.[6] In this study, the "three-points checklist" had the lowest sensitivity (68.9%). At the same time, the Menzies method had the highest sensitivity and the lowest specificity (95.1% and 24.8%, respectively). The "ABCDE rule" showed the highest specificity (59.4%), higher than the "Chaos and Clues" method (40.2%). From these last numbers, the large number of false positives is deduced. The bias of the clinical experience of those who performed the dermoscopy is also considered.[4]

We must consider, among other factors, the interaction between the dermatologist and the pathologist so that in lesions with high suspicion a more accurate microscope slide revision is conducted. The bias of histopathology becomes more evident when one does not know the pathologist who studies the microscope slide, and more than that, several pathologists analyze the samples of the study[4] in question, and the interpathologists subjectivity is a limitation of this study.

In this sample, we observed high frequencies of blue nevus/ Reed nevus that have common dermoscopic features (clues) with invasive melanoma (areas without bluish or blackened structures and peripheral pseudopods in the case of Reed nevus).

It should be emphasized that we did not include in the sample supposedly benign excised lesions nor did we perform a dermoscopic imaging test with medical evaluations of this service to quantify the sensitivity and the specificity since we do not have the possible false negatives of dermoscopy. It is known that very recent melanomas may not be discernible from nevi, hence the importance of early follow-up in at-risk patients or of excisions in cases of doubt.[7]

In the present study, a positive predictive value (PPV) of 19.35% was found for the diagnosis of melanoma. In the Ahnlide study, [17] a PPV of 51% was found for 108 excised melanocytic lesions. This same study also mentions Heal *et al.*, with a PPV of 33.3%, but points out that the prevalence of melanoma (results from Sweden and Australia, respectively) tends to increase this number.

CONCLUSION

In this study involving 62 lesions of clinical and dermatoscopic risk, we found 12 cases of melanoma, i.e., a positive predictive value of 19.35%. Rigorous dermoscopic follow-ups and the formation of risk scales may be alternatives to optimize the excision criterion. On the other hand, given the magnitude of the progression of melanoma, failing to make an early diagnosis can be fatal for the patient.

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Original Articles

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Comparative study on the use of systemic minocycline versus systemic corticosteroid therapy in the treatment of active vitiligo

Estudo comparativo do uso da minociclina sistêmica versus corticoterapia sistêmica no tratamento de vitiligo em atividade

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ABSTRACT

Introduction: Vitiligo is a chronic acquired skin disease, which evolves with depigmentation. The control of disease activity is a therapeutic challenge. Systemic corticosteroids, in daily use or in pulse doses, are the most used treatment for the disease.

Objective: To evaluate the effect of minocycline on the control over vitiligo activity compared with the corticosteroid therapy.

Methods: Randomized clinical trial with 16 active vitiligo vulgaris patients, divided into two groups: MINO group: minocycline 100mg/day, orally, for three months; and CORT group: prednisolone 0.3mg/kg/day, orally, for two months, and 0.15 mg/kg/day in the third month. The patients were evaluated before and after the treatment by photographic records and evaluation of the scores obtained by the VIDA score (vitiligo disease activity score).

Results: According to the photographic records, there was control of vitiligo activity in 100% of patients in the MINO group compared with 60% in the CORT group. In the comparison using the VIDA score, we noticed a statistically significant difference for both groups; however, we found that the reduction was greater in the MINO group, evidencing more effectiveness of minocycline in controlling the vitiligo activity.

Conclusion: This study demonstrated the efficacy of minocycline in the control of active vitiligo compared with a systemic corticosteroid regimen. Additional studies should be performed to confirm its efficacy.

Keywords: Vitiligo; Minocycline; Prednisolone

RESUMO

Introdução: *Vitiligo é uma doença cutânea adquirida crônica, que evolui com despigmentação. O controle da atividade da doença é um desafio terapêutico. Os corticosteroides sistêmicos, em uso diário ou sob a forma de pulsos, constituem o tratamento mais utilizado para a doença.*

Objetivo: *Avaliar o efeito da minociclina no controle sobre a atividade do vitiligo em comparação ao corticosteroide.*

Métodos: *Ensaio clínico randomizado com 16 pacientes com vitiligo vulgar em atividade, distribuídos em dois grupos: Grupo MINO - minociclina 100mg/dia, via oral, por três meses; e Grupo CORT - prednisolona 0,3mg/kg/dia, via oral, por dois meses e 0,15mg/kg/dia no terceiro mês. Os pacientes foram avaliados antes e depois do tratamento por: registros fotográficos e avaliação das pontuações obtidas pelo escore VIDA (escore de atividade da doença vitiligo).*

Resultados: *De acordo com os registros fotográficos, houve controle da atividade do vitiligo em 100% dos pacientes do Grupo MINO em comparação a 60% do Grupo CORT. Na comparação para o escore VIDA, notou-se diferença estatisticamente significante para ambos os grupos; porém, constatou-se que a redução foi maior no Grupo MINO, evidenciando maior efetividade da minociclina no controle da atividade do vitiligo.*

Conclusões: *Este estudo demonstrou a eficácia da minociclina no controle do vitiligo em atividade em comparação a um esquema de corticoterapia sistêmica. Estudos adicionais devem ser realizados para confirmar sua eficácia.*

Palavras-chave: *Vitiligo; Minociclina; Prednisolona*

INTRODUCTION

Vitiligo is a chronic acquired skin disease that evolves with depigmentation of the skin,[1,2] with no difference between race, gender or age.[3,4,5] The mean age of the disease onset is around 20 years.[1]

The clinical manifestations include achromic macules that initially may appear hypochromic, surrounded by skin with healthy or discreetly erythematous/ hyperchromic aspect. They usually occur in photoexposed areas such as the face, back of the hands, upper thorax and periorificial regions, as well as in hairs (leukotrichia), mainly pubic hair, eyebrows, and eyelashes.[1,3,4,6] The lesions tend to exhibit centrifugal growth and can affect any skin site, including mucous membranes. Local trauma (acute or chronic) can trigger the onset of vitiligo characteristic lesions on the skin that has not previously been affected (isomorphic phenomenon of Köebner).[3,6]

The exact etiology is still not clearly defined, but it is believed in the influence of immunological, neurological, biochemical and genetic factors, with emphasis on the latest advances in genomic studies.[3,4,7,8,9]

It is important to assess whether vitiligo is stable or progressive since management strategies differ in each case. The vitiligo is considered stable when it presents no progression for a period of one year or more [10,11] and, if the disease presents evolution, it is considered active. However, there are controversies according to the reference consulted.

The treatment of vitiligo should be individualized, depending on the extent and evolution of the condition and on the disease activity.

The available information on systemic vitiligo treatment is limited.[12] Recently, epidermal oxidative stress has been documented in patients with vitiligo, postulating that free radical-mediated damage acts as an initial pathogenic event in the degeneration of melanocytes.[13]

As one of the possible forms of systemic treatment currently under study, minocycline, an antimicrobial of the class of tetracyclines, presents anti-inflammatory and antioxidant effects in addition to its already well-established antimicrobial action.[14] Its mechanism of action has not been completely elucidated, but it involves inhibition of free radicals and production of cytokines, interference in protein synthesis, modulation of metalloproteinases action, and antiapoptotic action. There is evidence regarding the safety and efficacy of its use at the dose of 100 mg/day for three months.[14]

In one study, Song *et al.*[15] demonstrated that minocycline may spare melanocytes from oxidative stress *in vitro*. This study concluded that this drug protects melanocytes against apoptosis induced by H2 O2 (hydrogen peroxide) *in vitro*. [15]

In a prospective study involving 32 patients, Parsad and Kanwar [14] showed the efficacy of minocycline at the dose of 100 mg/day in controlling vitiligo disease activity. A more recent prospective study involving 50 patients concluded that dexamethasone in oral minipulse therapy (OMT) and oral minocycline are effective drugs to control vitiligo activity. [13]

In fact, topical and systemic corticosteroids are currently the standard treatment for active vitiligo. Corticosteroids administered systemically, daily or in the form of pulses, are efficient in controlling the disease activity.[16] Its use is based on the physiopathogenic theory of vitiligo autoimmunity,[17] and several studies demonstrate its effectiveness.[16,18,19,20,21,22]

However, the administration of systemic corticosteroids for prolonged periods may cause unacceptable adverse events. In patients with extensive or rapidly expanding vitiligo, Pasricha and Khaitan[16] reported that oral minipulse therapy with betamethasone has minimized adverse events. With the administration of a single dose of betamethasone/dexamethasone 5 mg orally after breakfast on two consecutive days per week, the authors reported 80% repigmentation and control of disease progression in 89% of 40 vitiligo patients, without serious adverse events.[16,21]

Other studies have shown the efficacy of low dose oral prednisolone in controlling vitiligo activity. Using daily doses of oral prednisolone of 0.3 mg/kg body weight, long-term treatment was possible, and adverse events were kept to a minimum.[18,21]

The objective of this randomized, open-label clinical trial in patients with clinical diagnosis of active vitiligo vulgaris was to compare the effect of minocycline on vitiligo activity with the conventional treatment, which is systemic corticosteroid.

MATERIALS AND METHODS

VIDA Score

First used in the study by Njoo *et al.* in 1999,[11] the vitiligo disease activity score (VIDA) consists of a scale that considers the presence of new lesions and/or the expansion of existing lesions and the period of evolution. Based on the patient's own reports, the VIDA score consists of a total of six points: VIDA score +4: activity lasting six weeks or less; score +3: activity lasting from six weeks to three months; score +2: activity lasting from three to six months; score +1: activity lasting from six to 12 months; score 0: stable disease for one year or more; score -1: stable disease with spontaneous repigmentation for one year or more. Lower VIDA score indicates less vitiligo activity.[10]

Population

Twenty-five patients were invited to participate in the study. Of these, 16 presented the eligibility criteria and were included. The study elected men and women between 14 and 65 years old with clinical diagnosis of active vitiligo vulgaris, defined as a vitiligo disease activity score (VIDA[11]) of +3/+4; regardless of the duration of prior therapy, who were able and willing to comply with all scheduling and visiting, treatment, and evaluation requirements, and were also able to understand and provide the informed consent.

None of the patients presented a history of decompensated comorbidity; immunosuppression; abnormal photosensitivity; active cancer; current infection; collagenosis; and benign intracranial hypertension (pseudotumor cerebri). Pregnant women, nursing mothers, and women who intend to become pregnant

during the course of the study were excluded; as well as patients on immunosuppressive drugs; concomitant use of other antimicrobials or who have shown hypersensitivity to tetracyclines.[23] Patients participating in other studies involving drugs or other devices in the three months prior to or during enrollment in this study were not admitted.

We conducted the history and clinical examination, including information such as age at vitiligo onset, duration of the disease, presence of family history or any other associated disease, triggering factors (emotional, physical or medication), evaluation of the percentage of the affected body surface, and presence of mucous membranes and hairs involvement.

Patients who received topical or systemic therapy for vitiligo were given a washout period of two and four weeks, respectively, before inclusion in the study.

This study protocol was submitted and approved by the Committee for Ethics in Research of the University of Mogi das Cruzes (SP) (CAAE 57065116.0.0000.5497).

Randomization and intervention

Randomly, the 16 patients selected were assigned to one of the two treatment groups, 11 for the MINO Group and five for the CORT Group, defined as:

MINO Group - Patients receiving minocycline 100 mg/day, orally, for three months.

CORT Group - Patients receiving prednisolone 0.3 mg/kg/day, orally, for two months, and 0.15 mg/kg/day in the third month of treatment.

No topical or phototherapy was allowed during the study period.

Endpoints

The patients were followed up for three months and were evaluated at baseline, after four weeks, to check adherence to the treatment, and after 12 weeks.

At baseline and at the end of the treatment, after 12 weeks, photographic records were made in the studio, with a Lumix Panasonic camera, with entire body images of the patients under study and of each vitiligo lesion.

The endpoints assessed were:

1) Assessment of disease activity, from the objective judgment of comparative pre and post-treatment photographic records, by two blinded medical examiners, to determine the stabilization or not after one of the treatments. The agreement test between the two experts showed no difference in terms of results.

Each isolated lesion was assessed in terms of its expansion and skin presentation in an overall way regarding the appearance of new lesions, classified as: - Stable, for stabilization of vitiligo; and – Unstable, for absence of stabilization. Also, the presence or absence of repigmentation in the study groups was evaluated;

2) Assessment of disease activity based on patients' reports, using the VIDA[11] questionnaire applied at baseline and at the 12th -week post-treatment.

Data analysis methodology

The following tests were used in the statistical analysis of the results: Wilcoxon test, Mann-Whitney test, Test for the Equality of Two Proportions, Fisher Exact Test, Confidence Interval for the Mean, P-value. We chose to use non-parametric tests since the data set had a low sampling rate (less than 30 subjects). In this statistical analysis, we used the software: SPSS V20, Minitab 16 and Excel Office 2010.[24, 25, 26]

RESULTS

Of the 16 patients selected for the study, the total number of individuals who completed the proposed treatment for the MINO Group was 11 patients; and, for the CORT Group, five patients. A subject who has completed the study is defined as a patient enrolled in accordance with the inclusion/ exclusion criteria and who attended the initial visit, the follow-up visit, and the visit immediately after the end of the treatment.

A significance level of 0.05 (5%) was defined for this study. All confidence intervals throughout the study were constructed with 95% statistical confidence.

The mean age of patients in the MINO Group was 42.6 years and in the CORT Group was 36.4 years. It was found that in both groups the age variability was low because the coefficient of variation was less than 50%, demonstrating that the data are homogeneous.

The percentage distribution of men and women between the study groups was: 81.8% of women and 18.2% of men in the MINO Group; 60% of women and 40% of men in the CORT Group.

After three months of treatment, by analyzing the pre and post-treatment comparative photographic records, conducted by two blind medical examiners, we verified that there was control of vitiligo activity in 100% of patient in the MINO Group. In the CORT Group, 60% of the patients evolved with activity control (Chart 1).

Regarding the analysis of the VIDA score, a statistically significant difference was observed for both groups regarding the control of vitiligo when the moments before and after treatment were compared. In the MINO Group, the mean score was reduced from 3.27 to 1.64 (p-value=0.002), while in the CORT Group it decreased from 3.60 to 3.20 (p-value=0.157) (Chart 2). This analysis was performed by group, using the Wilcoxon test.

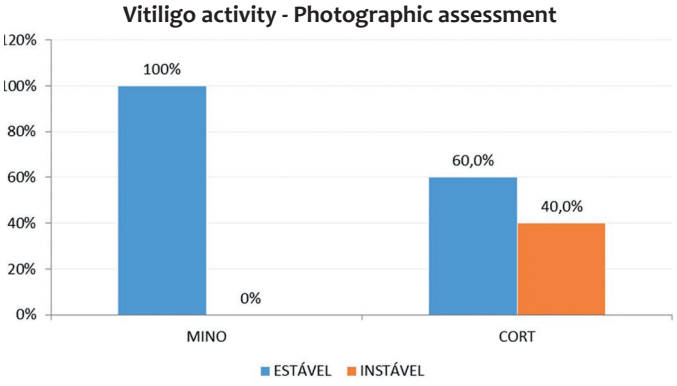
We also analyzed the groups in the gain, i.e., in the simple difference between the mean of the results of post minus pre-treatment of the VIDA questionnaire data, using the Mann-Whitney test and concluding that there was a statistically significant difference between the groups. The reduction, however, was higher in the MINO Group: -1.64 *versus* -0.40 in the CORT Group (p-value=0.004), showing higher effectiveness of minocycline in the control of vitiligo activity in this group of patients (Graph 3).

In some patients in the MINO Group, in addition to vitiligo stabilization, it was observed repigmentation at the end of the third month of treatment, as exemplified in Figures 1 and 2.

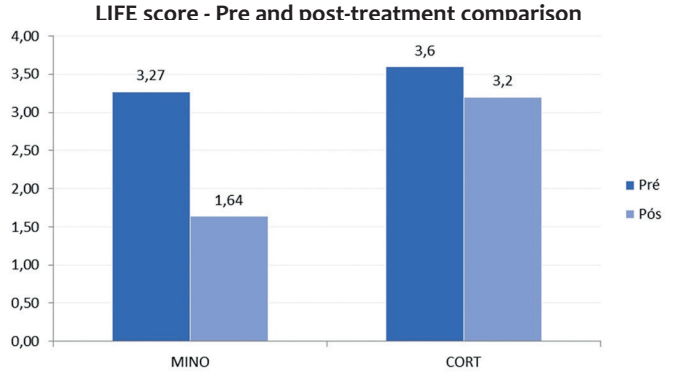
Table 1 presents the distribution regarding repigmenta-

tion between MINO and CORT Groups, showing, as additional data, repigmentation in 54.5% of the patients at the end of the treatment with minocycline, compared with 20% of the patients in the prednisolone group.

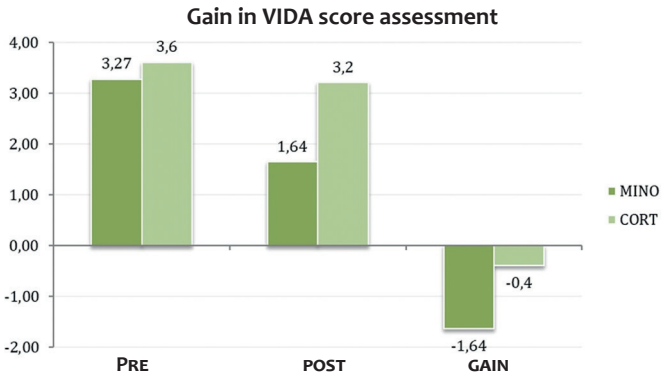
No patient presented severe adverse events during the study, and these were most often attributed to the use of corticosteroids. Only two of the 11 patients reported adverse events associated with the use of minocycline (vaginal discharge and vertigo), as shown in Table 2.



Graph 1: Vitiligo activity: assessment of photographic records after treatment



Graph 2: Pre and post-treatment comparison by group for the VIDA score



Graph 3: Gain = comparison of the simple post and pre-treatment difference in VIDA score assessment between groups

DISCUSSION

Vitiligo is a skin depigmentation disorder resulting from a selective loss of melanocytes. Both sexes are equally affected, and there are no apparent differences in occurrence rates according to phototype or race. [27]

It is a complex disease, associating genetic aspects and environmental factors with metabolic and immune changes.[27]

Aiming to expand the therapeutic arsenal, the present study intended to evaluate the effect of minocycline on vitiligo activity compared with the conventional treatment, which is the corticosteroid.

Minocycline has a broad range of anti-inflammatory and immunomodulatory actions in addition to its already well-characterized antimicrobial effect.[14] Its mechanism of action is complex and not fully understood, including inhibition of free radical and cytokine production, interference with protein synthesis, modulation of the metalloproteinases action, and antiapoptotic action.[14] Both oxidative stress and apoptosis have been shown to play a significant role in the pathogenesis of vitiligo. Thus, minocycline offers a potentially powerful approach to controlling disease activity.[14]

In a previous study, Parsad and Kanwar[14] evaluated the efficacy of minocycline 100 mg once daily in 32 patients. The

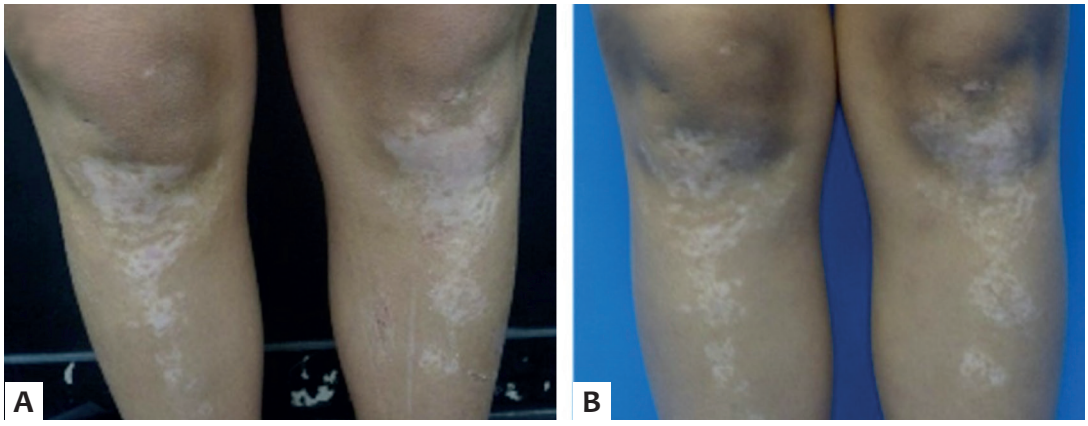


Figure 1: (A) Pré e (B) pós-tratamento com minociclina

study showed control in the disease progression in 29/32 patients and only three patients presented the development of new lesions and/or increase of existing lesions. Ten patients had discontinuation of depigmentation after four weeks of treatment. Also, seven patients presented moderate to severe repigmentation.

Recently, Singh *et al.*[13] conducted a randomized controlled trial to evaluate the efficacy of dexamethasone oral minipulse therapy *versus* oral minocycline therapy in patients with active vitiligo vulgaris. They observed that of the 25 patients in the minocycline group, only six (24%) developed new lesions during 24 weeks of follow-up, while in the dexamethasone oral minipulse therapy only three (12%) patients presented disease activity. These results in the minocycline group were comparable to those observed in the Parsad and Kanwar study.[14]

The present study demonstrated that in both treatments, the MINO Group and the CORT Group showed control in the vitiligo activity with statistical significance assessed by the VIDA questionnaire. However, comparing the groups at each time of treatment (pre and post) and also evaluating the gain (simple difference between the mean of the results of post minus pre-treatment of the VIDA score), it was observed the existence of statistically significant difference among the groups assessed, pointing to better effects with the minocycline than with the corticosteroid treatment.

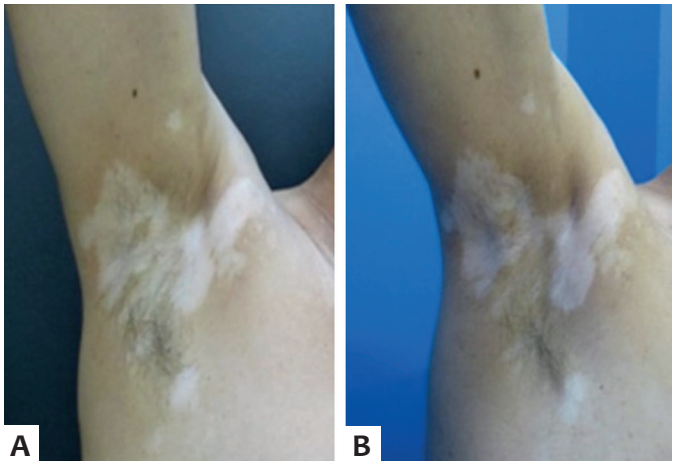


Figure 2: (A) Pre and (B) **post-treatment with minocycline**

Table 1: Distribution of "repigmentation"

Repigmentation	Group MINO		Group CORT	
	N	%	N	%
No	5	45.5%	4	80%
yes	6	54.5%	1	20%
P-value	0.670		0.058	

Table 2: Distribution of adverse events

Adverse event	Group MINO			Group CORT		
	N	%	P-value	N	%	P-value
Vaginal discharge	1	9.1%	0.114	0	0%	0.114
Edema	0	0.0%	0.490	1	20%	0.490
Face and abdomen edema	0	0.0%	0.490	1	20%	0.490
Epigastric pain	0	0.0%	0.490	1	20%	0.490
Vertigo	1	9.1%	0.114	0	0%	0.114
No	9	81.8%	Ref.	2	40%	Ref.

Analyzing the variables repigmentation and vitiligo stabilization in the treatment groups, it was observed that there is no statistical relationship between the data, i.e., they are independent variables.

The observed adverse events were, in a descriptive way, in the MINO Group: a patient with vaginal discharge and a patient with vertigo; and in the CORT Group: three of the five patients reported adverse events, being two patients with edema and one patient with epigastralgia. Studies with a more significant sample may be necessary to better evidence the results of the use of oral minocycline in the treatment of vitiligo.

Thus, the use of minocycline as a potential medication to control active vitiligo would be a therapeutic option with less severe adverse events compared with prolonged use of systemic corticosteroids.

CONCLUSION

The present study demonstrated the efficacy of minocycline in the control of active vitiligo compared with the treatment already established with systemic corticosteroid therapy, with an already well-established activity control effect. According to the judgment of the comparative photographic records before and after three months of treatment, the vitiligo activity was controlled in 100% of the patients in the MINO Group, while in the CORT Group the control of the activity was 60%.

Regarding the VIDA score, a statistically significant difference was observed for both groups in the disease control; however, it was found that the reduction was greater in the MINO Group, evidencing more effectiveness of minocycline in the control of vitiligo activity. Further controlled studies should be conducted to confirm its efficacy.

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Original Articles

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Anhydrous fluid serum as vehicle for drug delivery formulations: sterility test results for bacterial and fungal growth

Sérum anidro fluido como veículo para formulações de drug delivery: resultados do teste de esterilidade para crescimento bacteriano e fúngico

DOI: http://dx.doi.org/10.5935/scd1984-8773.20191121340

ABSTRACT

Introduction: Topical delivery of drugs is essential in Dermatology. Due to the difficulty of permeation of the stratum corneum, drug delivery techniques have been highlighted. The use of non-specific formulations for this purpose makes raises the concern of possible adverse events and the microbiological safety of these formulations.

Objective: To assess bacterial and fungal growth in anhydrous fluid serum through simple sterility test.

Materials and methods: The simple sterility test was performed on an anhydrous serum containing lipophilic and hydrophilic active substances. This test was performed three months after the manufacture of the product.

Results: The formulation studied was approved in the simple sterility test conducted three months after the manufacture of the product, even without the use of preservatives in the formulation.

Discussion: The assessed formulation was approved in the sterility test possibly due to the fact that the serum vehicle has mineral and anhydrous origin, characteristics that do not favor the proliferation of microorganisms.

Conclusions: Although only the vehicle counting specific assets has been tested, the results of this study are promising and demonstrate the need for future studies broadly encompassing this subject.

Keywords: Drug Administration Routes; Brazilian Pharmacopeia; Skin Cream

RESUMO

Introdução: *A entrega tópica de medicamentos é essencial na Dermatologia. Devido à dificuldade de permeação do estrato córneo, as técnicas de drug delivery vêm recebendo destaque. O uso de formulações não específicas para este fim nos faz atentar para possíveis efeitos adversos e para a segurança microbiológica destas formulações.*

Objetivo: *Avaliar crescimento bacteriano e fúngico no sérum anidro fluido por meio do teste de esterilidade simples.*

Materiais e métodos: *O teste de esterilidade simples foi realizado em um sérum anidro contendo ativos lipofílicos e hidrofílicos. Este teste foi realizado três meses após a manufatura do produto.*

Resultados: *A formulação estudada foi aprovada no teste de esterilidade simples realizado três meses após a manufatura do produto, mesmo sem uso de conservantes na formulação.*

A formulação em estudo foi aprovada no teste de esterilidade possivelmente devido ao fato de o veículo sérum ser de origem mineral e anidra, características que não favorecem a proliferação de micro-organismos.

Conclusões: *Embora somente o veículo contando ativos específicos tenha sido testado, os resultados deste estudo são promissores e demonstram a necessidade de estudos futuros que englobem de forma mais ampla o assunto.*

Palavras-chave: *Vias de administração de medicamentos; Farmacopeia brasileira; Creme para a pele*

INTRODUCTION

Topical delivery of drugs in Dermatology is key to successful effectiveness. Medicines and active principles applied to the skin need to penetrate and reach a target structure. The epidermis' barrier function is maintained by the stratum corneum's double lipid layer, which is the main limiting factor for topical delivery of medications. [1] Drug penetration through untouched stratum corneum occurs by diffusion and via cutaneous appendages to a lesser extent. In addition, only lipophilic molecules smaller than 500da are able penetrate it, and only 1% to 5% of the substances applied to the skin are effectively absorbed and become bioavailable. [2] The drug delivery technique corresponds to using chemical, mechanical or physical methods aimed at optimizing the penetration of drugs. [2] The use of non-specific formulations for drug delivery can lead to undesirable effects, such as irritative and allergic dermatitis, foreign body granulomas, and cutaneous infection. [3]

Many cosmetic formulations available on the market contain chemical preservatives and additives in their formulation, making them unsuitable for use in drug delivery. Moreover, contamination of the formulations by microorganisms may occur, making them unsuitable for this purpose. [4]

MATERIALS AND METHODS

The growing demand for adequate formulations for drug delivery that can be applied immediately after medical dermatological procedures gave motivation the authors of the present paper to perform a sterility test on the anhydrous serum vehicle that is claimed to have properties that promote the safe delivery of the medication containing the following lipophilic and hydrophilic active principles: 6% hydroxyprolisilane® (increases synthesis of collagen and elastin, improves healing), 4% MDI Complex® (anti-inflammatory, reduces erythema and edema), 1% PBR® (re-epithelizing properties), 0.1% madecassoside (inhibits a series of inflammatory cytokines, has regenerative action and stimulates collagen I), and 4% panthenol (promotes healing and regeneration) Table 1-Farmatec® Pharmacy, Porto Alegre (RS), Brazil).

The formulation was tested for its ability to remain sterile – including after the vial had been opened – simulating what occurs when the product is applied by a physician at a practice and / or is used by the patient at home.

The simple sterility test was performed as described in the Brazilian Pharmacopoeia - 5th Edition (Farmacopeia Brasileira - 5ª edição, item 5.5.3.2 for sterile products) (Pharmacontrol - quality control laboratory, Porto Alegre RS, Brazil). The same test is applied for sterile injectable and topical formulations, having been performed three months after the sample was manufactured. In addition, the vial was opened and used during a three-month period with a view to emulate conditions of use at the physician's practice. The test employs two culture mediums, which are previously tested to ensure their capacity to promote bacterial growth by directly inoculating two units of the manufactured 4.5 ml batch (corresponding to 5% of the total batch): (i) liquid medium of 1-thioglycollate for aerobic and anaerobic bacteria and (ii) fluid soybean-casein medium for aerobic bacteria, yeasts and fungi. The anhydrous serum – the substance studied in the present paper – remained incubated for a period of 14 days. After the first tested vial passes the sterility test, the batch's sterility is confirmed by incubating all manufactured vials in the culture mediums for 14 days, under the conditions described above. No microbial growth should occur. No active microbial growth should take place, and its occurrence corresponds to failure in passing the sterility test.

RESULTS

The sterility test confirmed the absence of microbiological contamination for bacteria, fungi and yeasts (Figure 1). Therefore, the studied formulation was approved in the sterility test, even without the presence of preservatives and three months after having been manufactured.

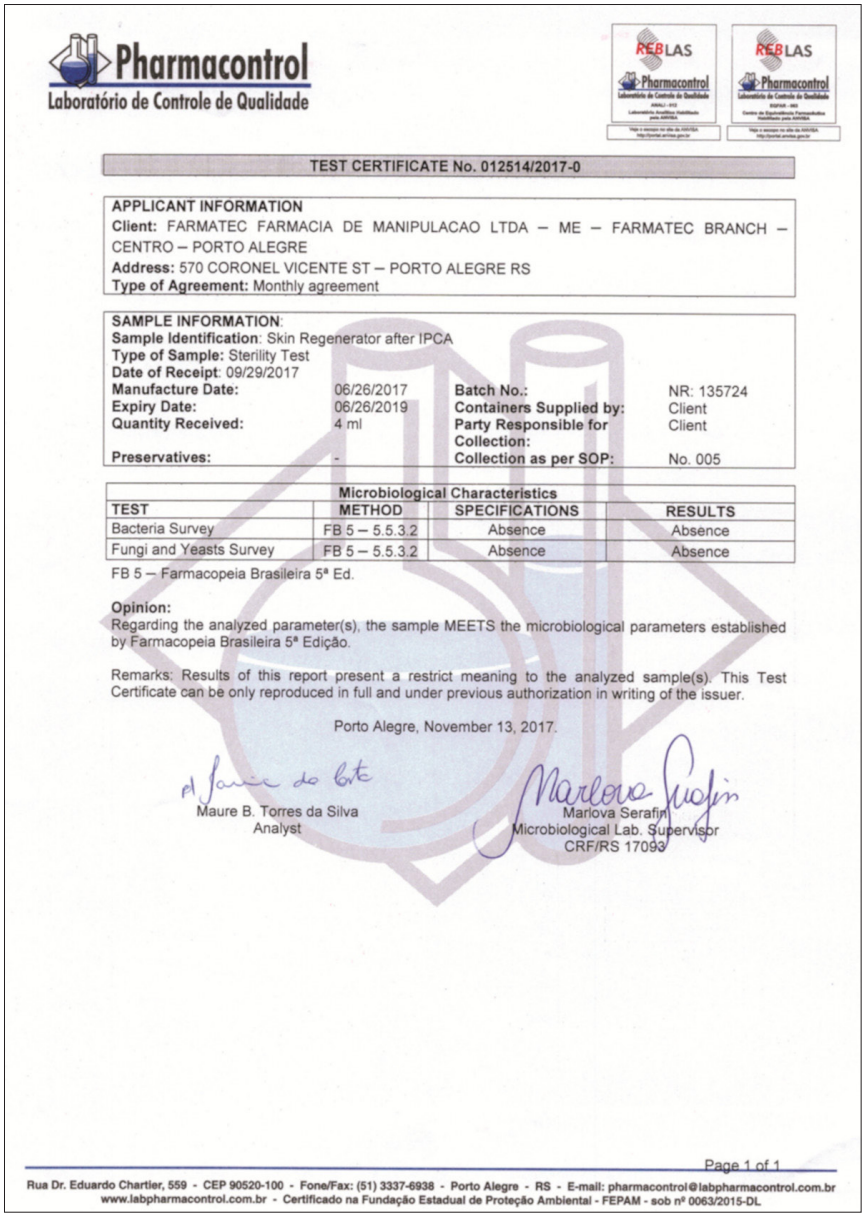
DISCUSSION

Choosing the right vehicle for drug delivery is crucial: creams, lotions, gels, and some serums are not adequate due to their high viscosity, which decreases transepidermal / transdermal delivery of active ingredients. [4] The ideal formulation for drug delivery should be devoid of and repel water, as well as being mineral in order to provide a barrier against transepidermal water loss (TEWL). In addition, it should favor healing and dermal permeation of hydrophilic and lipophilic substances. Furthermore, the formulation's pH should resemble that of the skin, give that a higher pH may lead to skin irritation and rupture of the cutaneous "acid mantle". [5] The anhydrous vehicle does not cause burning sensation and does not require the addition of preservatives and chemicals, theoretically offering microbiological safety related to bacterial and fungal proliferation. Moreover, the anhydrous serum promotes occlusion. [6] It is worth to note that, regardless of the drug delivery technique chosen to optimize drug permeation, occlusion is beneficial to increase the efficacy of the method, since it delays the recovery of the cutaneous barrier, leaving the stratum corneum permeable for longer. [7]

The authors believe that the studied formulation's successful outcome in the sterility test is linked to the fact that the vehicle is of mineral and anhydrous origin, which are features that do not allow the proliferation of microorganisms, which in turn favor the hypothesis that the formulation is safe for drug delivery, even if used immediately after ablative procedures.

Table 1: Baseline characteristics of the sample

Active ingredient	Active ingredient
Hydroxyprolisilane®	Hydrophilic
MDI Complex®	Hydrophilic
PBR®	Lipophilic
Madecassoside	Lipophilic
Panthenol	Hydrophilic



Pharmacontrol
Laboratório de Controle de Qualidade

REBLAS Pharmacontrol

TEST CERTIFICATE No. 012514/2017-0

APPLICANT INFORMATION
Client: FARMATEC FARMACIA DE MANIPULACAO LTDA – ME – FARMATEC BRANCH – CENTRO – PORTO ALEGRE
Address: 570 CORONEL VICENTE ST – PORTO ALEGRE RS
Type of Agreement: Monthly agreement

SAMPLE INFORMATION:
Sample Identification: Skin Regenerator after IPCA
Type of Sample: Sterility Test
Date of Receipt: 09/29/2017

Manufacture Date:	06/26/2017	**Batch No.:**	NR: 135724
Expiry Date:	06/26/2019	**Containers Supplied by:**	Client
Quantity Received:	4 ml	**Party Responsible for Collection:**	Client
Preservatives:	-	**Collection as per SOP:**	No. 005

Microbiological Characteristics			
TEST	**METHOD**	**SPECIFICATIONS**	**RESULTS**
Bacteria Survey	FB 5 – 5.5.3.2	Absence	Absence
Fungi and Yeasts Survey	FB 5 – 5.5.3.2	Absence	Absence

FB 5 – Farmacopeia Brasileira 5ª Ed.

Opinion:
Regarding the analyzed parameter(s), the sample MEETS the microbiological parameters established by Farmacopeia Brasileira 5ª Edição.

Remarks: Results of this report present a restrict meaning to the analyzed sample(s). This Test Certificate can be only reproduced in full and under previous authorization in writing of the issuer.

Porto Alegre, November 13, 2017.

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Page 1 of 1

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www.labpharmacontrol.com.br - Certificado na Fundação Estadual de Proteção Ambiental - FEPAM - sob nº 0063/2015-DL

Figure 1:
Simple sterility test results: the formulation was approved in the simple sterility test performed three months after the product had been manufactured

CONCLUSION

Choosing the right vehicle is crucial regardless of the method being used to promote drug delivery. For this reason and aiming at ensuring procedural safety, studies on specific vehicles for drug delivery are of great interest. Although only the anhydrous serum vehicle containing hydroxyprolisilane®, MDI Complex®, PBR®, madecassoside and panthenol has been tested, the outcomes observed in the present report are promising and can serve as a foundation for more comprehensive studies on the subject in the future.

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Diagnostic imaging

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Conflict of interests: None

Reflectance confocal microscopy as a tool for assessing the injury caused by microneedling: a serie of five cases

Microscopia confocal de reflectância como ferramenta para avaliar os efeitos causados pelo microagulhamento: uma série de cinco casos

DOI: http://www.dx.doi.org/10.5935/scd1984-8773.201911202

ABSTRACT

Microneedling is an ambulatory surgical procedure that can be used for different indications with the objective of stimulating the production of collagen. Five cases were evaluated in the first 72 hours after the procedure by reflectance confocal microscopy in order to evaluate the pores lifetime.

Keywords: Collagen; Wound healing; Ambulatory surgical procedures

RESUMO

O microagulhamento é um procedimento cirúrgico ambulatorial que pode ser utilizado para diferentes indicações com o objetivo de estimular a produção de colágeno. Foram avaliados 5 casos no transcorrer das 72 horas após o procedimento, por meio da Microscopia Confocal de Reflectância, com o objetivo de avaliar a vida útil dos orifícios.

Palavras-chave: *Colágeno; Cicatrização; Procedimentos cirúrgicos ambulatoriais*

INTRODUCTION

Microneedling has been used as minimally invasive technology for the treatment of various dermatological conditions such as acne scars, stretch marks and skin rejuvenation. [1], [2] It has also been applied aimed at increasing the absorption of drugs via transdermal route, creating pores in the epidermis and papillary dermis. [3], [4] Nevertheless, few studies have evaluated its initial effects within the epidermis and dermis. Therefore, the authors of the present paper have studied a series of cases through confocal reflectance microscopy (CRM), which is an *in vivo* auxiliary examination that allows the visualization of different levels of the skin with histological resolution. [5]

FIVE CASE REPORTS

A total of five patients with acne scars and skin photoaging who have signed a Free and Informed Form of Consent were included in the present study. Confocal reflectance microscopy images were acquired through a laser scanning confocal microscope with reflection close to that of the infrared (Vivascope 3000®, Caliber I. D, Rochester, NY, US), from the *stratum corneum* to the papillary dermis (horizontal cuts), at the right temple. One hour after of topical anesthesia had been applied (Pliaglis®, Galderma, São Paulo, SP, Brazil), microneedling was performed with the assistance of the Derma Roller device (Fabinject Technology, Taubaté, São Paulo, Brazil). The device had 540 microneedles with 1.5mm in length and was applied in the whole face aimed at causing punctate bleeding on the total facial area. The application region was evaluated by CRM immediately after (T0), 24 hours after (T1), 48 hours after (T2) and 72 hours after (T3) the procedure. All patients were advised not to apply any topical cream to the facial skin between CRM assessments. The CRM evaluation conducted at T0 evidenced a black linear cleft extending from the top of the epidermis to the papillary dermis – being more triangular in the dermis (Figure 1) – in all cases. At T1 and T2, the cleft has become a black circular structure in the upper epidermis (stratum corneum), in the epidermis and in the dermo-epidermal junction. Some of them contained a mild and bright substance. In the dermis, these black areas presented bright particles at T1 (Figure 1).

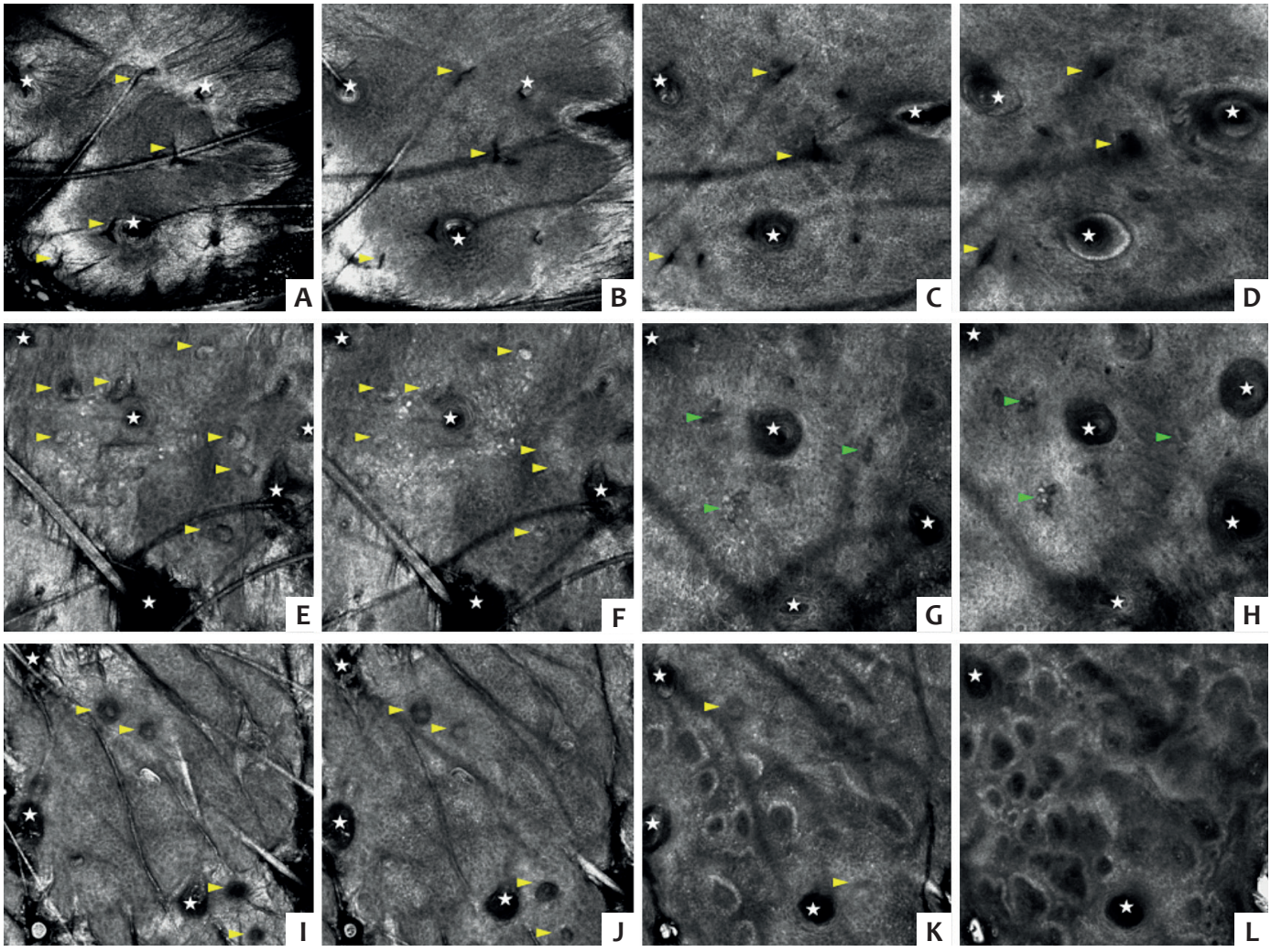

Figure 1: CRM images (500x500µm).
A, B, C and D. T0: Stratum corneum, supra-basal layer, dermoepidermal junction and papillary dermis, respectively, showing black fissures (yellow arrowheads) and follicular openings (white stars).
E and F. T1: Supra-basal and suprabasal/dermoepidermal junction layers, respectively showing circular black structures containing a light and substance (yellow arrowheads) and follicular openings (white stars).
G and H. T1: Dermoepidermal/papillary dermis layers and papillary dermis showing black areas with bright particles (green arrowheads) and follicular openings (white stars).
I, J and K. T2: Stratum granulosum, suprabasal layer and dermoepidermal junction, respectively, showing circular black structures containing a bright substance (yellow arrowheads) and follicular openings (white stars).
L. T2: Papillary dermis with absence of orifices, showing follicular openings (white stars).

DISCUSSION

Microneedling has been increasingly used in dermatology for cosmetic reasons due to its easy application technique and rare complications. It also appears promising for drug delivery since the stratum corneum is the major barrier for transdermal drug delivery and can be punctured by microneedles that mechanically pierce the skin layers leading to the transdermal absorption of the drug. [3, 4]

Using a new technology (CRM), the present study allowed the observation of perforations in the skin resulting from microneedling. The presence of the orifices in the epidermis and dermis, possibly increasing the skin's permeability – which is essential for the concept of transdermal drug release – is noticed immediately after microneedling (T0). The presence of a mild and bright substance in the epidermis' pores at T1, T2 and T3 may correspond to local subclinical inflammation responsible for micropore occlusion. This physiological process is not yet known, however it is believed that the micropore may close in a matter of hours. [3], [4] The finding of black areas with bright particles inside the papillary dermis allows the authors of the present paper to hypothesize whether it could correspond to inflammation caused by the micro injuries, leading to neovascularization and neocollagenesis, involved in skin rejuvenation. [4]

CONCLUSION

Finally, little is known about the useful life of the orifices and the injury caused by the treatment with microneedles. Therefore, the authors of the present article believe that CRM may be useful for unprecedented visualization of microneedling's mechanical and inflammatory events.

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A combination approach to treating acne scars in all skin types: carbolic CROSS, blunt bi-level cannula subcision, and microneedling

Abordagem combinada para o tratamento de cicatrizes de acne em todos os tipos de pele: CROSS com fenol, subcisão com cânulas em dois planos e microagulhamento

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ABSTRACT

Acne is a common condition that often results in scarring. We propose a novel treatment of acne scarring using: 1- chemical reconstruction of skin scars (CROSS), mainly with carbolic acid, 2- blunt bi-level cannula subcision, and 3- microneedling. A total of 139 patients were treated from 2017-2018, of which 89 (64%) were Fitzpatrick Skin Types IV-VI. This triple approach to treating acne scars resulted in consistent high satisfaction from patients and photographic evidence of improvement. A combination of CROSS (to stimulate neocollagenesis), subcision (to release dermal connective tissue tethering), and microneedling (to stimulate neocollagenesis) is effective for acne scar treatment.

Keywords: Acne vulgaris; Acne keloid; Cicatrix; Cosmetic techniques

RESUMO

A acne é uma condição comum que muitas vezes resulta em cicatrizes. Propomos um novo tratamento para as cicatrizes da acne usando: 1- reconstrução química de cicatrizes (CROSS), principalmente com fenol, 2- subcisão com cânula em dois níveis, e 3- microagulhamento. Um total de 139 pacientes foram tratados em 2017 e 2018, dos quais 89 (64%) eram de Fototipos de Fitzpatrick IV-VI. Esta abordagem tripla para o tratamento de cicatrizes de acne resultou em consistente alta satisfação dos pacientes e evidência fotográfica de melhoria. A combinação de CROSS (para estimular a neocolagênese), subcisão (para liberar as traves do tecido conjuntivo dérmico) e microagulhamento (também para estimular a neocolagênese) é eficaz para tratar as cicatrizes da acne.

Palavras-chave: *Acne vulgar; Acne queloide ; Cicatriz; Técnicas cosméticas*

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INTRODUCTION

Acne is a common multifactorial condition that affects up to 80% of girls and 90% of boys in the adolescence. Unfortunately, many people with acne end up developing scars – which causes cosmetic discomfort – with 30% of those affected considering this condition a major problem. [1] A number of medical and surgical approaches have been proposed for the treatment of these scars, including laser based resurfacing, chemical peels, radiofrequency, subcision, microneedling and others. Although various monotherapies may be useful, combined treatments can be more effective. In the present article, the authors propose a new treatment for acne scars using a multi-modality approach.

METHODS

A retrospective review was carried out with the medical records of all acne scar patients who were treated with combination therapies from January 2017 to December 2018. All patients were treated with a combination of three procedures: (i) chemical reconstruction of scars (Chemical Reconstruction of Skin Scars - CROSS), mainly with carbolic acid, (ii) blunt bi-level cannula subcision and (iii) microneedling. Each of these steps is described in more detail below.

Step 1) Carbolic CROSS: 88% carbolic acid was used to treat ice pick and box scars in a similar method to trichloroacetic acid (TCA) CROSS however with two main differences. Trichloroacetic acid CROSS was performed using 60-90% TCA applied with a toothpick into the middle of the scars, avoiding spillage onto the scar's shoulders. However, carbolic CROSS was performed with a very fine paint brush instead of toothpick due to the fact it was technically easier to fill the inside of these scars than with this method. In addition, the carbolic acid was allowed to spill slightly out onto the shoulder of the scar in order to soften the scar's shoulder and improve blending with unscarred skin. This was applied after degreasing with acetone and before applying local anesthesia for the subcision procedure.

Step 2) Subcision: The initial subgroup of patients underwent standard subcision performed with a Nokor 18 gauge needle. Indirect lighting was used to visualize the patients' elevated and atrophic scars, and to determine the area to be subscised. After marking, the area was tumesced with 1% lidocaine mixed with sodium bicarbonate in a 2:1 ratio, using a 3cc or 5cc syringe and a 1" 25 or 30 gauge needle. Approximately 18-24cc of this diluted lidocaine mixture was used per cheek. For subcision with Nokor, an 18-gauge needle was used to create multiple puncture sites.

Alternatively, a second subset of patients was treated with multilevel subcision using a 70mm, 18-gauge cannula, which only required one puncture site. This bi-level subcision was performed parallel to the skin, aiming directly underneath the skin and breaking up the scar tissue, or aiming more towards the dermal fat junction, breaking scar tissue and adhesions and producing audible cracking sounds while doing this. A slow piston movement was used, moving the cannula back and forth and in a fanning pattern. During tumescence, the indents produced by the acne scar tethers were clearly visible. The goal was to achieve very little resistance in the subscised area.

Step 3) Microneedling: Microneedling was performed with the Collagen PIN® device (Induction Therapies, Louisville, US) aimed at triggering percutaneous collagen induction immediately after the subcision. This device uses a disposable tip with 36 needles and revolves at 1,200 RPM. The device was used with a stamping technique, holding the tip on the skin for approximately 2-3 seconds (effectively producing 400-600 needle punctures) before moving on to the adjacent skin. The endpoint was punctate bleeding, and as such, appropriate depth of the needling was varied according to both facial region (temples are thinner) and the individual patient's skin thickness. The needle depth was calibrated from 1.5-2.5mm deep in the cheeks and 0.5mm on the temples and forehead.

Further treatment of the patient included Aquaphor® (Eucerin, US) and / or hyaluronic acid gel (HA). After five days, some patients also used vacuum suction (over-the-counter sales device, popular on many blogs about acne scars) aimed at reducing tether reattachment.

RESULTS

A total of 139 patients were treated. Of those, 89 (64%) had Fitzpatrick IV-VI skin phototype. Shadow-lit before and after photos were used to assess changes, along with patient feedback on side effects and satisfaction level. Patients received on average two treatments each (range = 1-4). This triple approach to the treatment of acne scars resulted in consistently high patient satisfaction as well as photographic evidence of improvement (Video 1). Typical adverse events included hematomas (from the subcision), small crusts and desquamation (from the CROSS and microneedling), and edema (from the anesthesia and subcision). There were rare cases of post-inflammatory hyperpigmentation (PIH).

Subcisions carried out with the cannula caused much less bleeding and subsequent hematoma formation as compared to the Nokor subcision. In addition, the cannula based subcision could be performed safely in the temples and marionette regions. The Nokor needle was not used on the temples or on the marionette region, due to the risk of injuring the blood vessels, being therefore used only in the cheeks. Patients who had both Nokor and cannula subcision consistently reported experiencing less severe side effects after cannula subcision. Specifically, patients who underwent both types of subcision tended to develop hematomas after Nokor subcision but not after cannula subcision.

Video 1: Procedural video including CROSS with carbolic acid, subcision with cannula and microneedling for treatment of acne scars.
The video is available on the Journal's website

DISCUSSION

The authors of the present study describe a technique that combines carbolic CROSS, subcision and microneedling for the treatment of acne scars. All patients experienced improvement in their acne scarring and were satisfied with the results.

Many patients had a history of ablative fractionated CO2 resurfacing, having experienced little or no improvement from that single-modality treatment. This novel triple procedure is characterized by greater patient satisfaction, fewer adverse events and shorter healing time. In addition, the specific combination of procedures can be adjusted for each individual patient according to the required recovery time or skin tone and scarring patterns. [2]

Carbolic acid was chosen over ATA for the CROSS in the first step of this combined procedure due to the fact that ATA is a highly penetrating agent and may cause extensive scars. In contrast, carbolic acid is a vesicant, creating edema and then a very superficial vesiculation of the skin that lines the acne scar. This tends to lead to better results and lower risk of enlarged scars. [3]

For the second step of this combined procedure, a Nokor or cannula based subcision was specifically used to release papillary scars from the dermis and deeper tissues. This controlled destruction of fibrous scar tissue leads to trauma and regeneration of collagen in the area. [4] Multiple passes may be required to completely release tethered icepick type scars.

Microneedling was used in the third step of this combined procedure. Previous clinical and histological studies have demonstrated the effectiveness of microneedling, specifically for boxcar and rolling scars. Histology shows an increase in the thickness of the epidermis, type I, III and VII collagen, elastin and tropoelastin after microneedling of acne scars. However, deep icepick or atrophic scars with tethered scarring below the skin did not respond as well as boxcar-type and rolling scars, probably due to the microneedling's inability to release these fibrous connections. Therefore, the addition of subcision in our triple combined treatment would resolve this limitation of microneedling by first releasing the fibrous connective tissue adhering to icepick-type scars.

CONCLUSION

This triple combination of procedures can be used to treat all skin types and can have greater efficacy and lead to adverse events that are less severe than those caused by the previous methods. Further studies are recommended.

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Case Reports

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Lentigo maligna of the eyelid: management with Dermatology-Oculoplastic interface: for the purpose of a case

Lentigo maligno palpebral: o manejo com a interface Dermatologia-Oculoplástica: a propósito de um caso

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ABSTRACT

A 74-year-old male patient came to medical consultation presenting a hyperpigmented stain near the lower left eyelid edge, with varying colors, between different shades of brown; the biopsy confirmed the diagnosis of lentigo maligna. Its location on the eyelid edge is rare. There is some divergence in the literature regarding the best method for its treatment. Non-surgical therapeutic options, such as imiquimod, have been suggested, as well as different variants in surgical management and required margins. In the case described, it was chosen to manage with conservative margins, preserving the functionality of the area.
Keywords: Lentigo; Melanoma; Eyelid Neoplasms; Dermoscopy; Pathology, Surgical; Surgery, Plastic

RESUMO

Apresentou-se à consulta um paciente masculino de 74 anos, portador de uma mancha hiperpigmentada junto à borda palpebral inferior esquerda, com cores variadas, entre diferentes matizes de marrom; a biópsia confirmou o diagnóstico de lentigo maligno. A sua localização na borda palpebral é rara. Há alguma divergência na literatura quanto ao melhor método para seu tratamento. Opções terapêuticas não cirúrgicas, como o imiquimode, têm sido apresentadas, bem como diferentes variantes no manejo cirúrgico e nas margens requeridas. No caso descrito, optou-se por manejo com margens conservadoras, preservando-se a funcionalidade da área.
Palavras-chave: Melanoma; Lentigo; Neoplasias Palpebrais; Dermoscopia; Patologia Cirúrgica; Cirurgia Plástica

INTRODUCTION

The lentigo maligna melanoma, an *in situ* lesion commonly found on the face and cervical region, may compromise sites where treatment is challenging, such as the palpebral region. Lentigo maligna in this location is rare, with rates less than 1% among melanomas and accounting for up to 1% of all palpebral malignancies.[1,2] Lesions with conjunctival involvement characteristically have more aggressive behavior. The prognosis is generally good, however recurrences are frequent, depending on the treatment used. Considering its relative low aggressiveness and delicate topography, this type of melanoma's management persists as debatable. Many authors advocate the use of non-surgical therapies, even though potentially superior recurrence rates are considered.[1,2] The authors of the present study describe the case of a patient whose lesion was located along the lower eyelid border, with excellent oncological, functional and aesthetic outcomes after surgical treatment.

CASE REPORT

A 74-year-old male patient attended a medical consultation complaining of a darkened lesion on the left lower ciliary border two years before, with progressive growth. He had previously been seen by an ophthalmologist due to bilateral entropion, with prescription of artificial tears eye drops and extraction of eyelashes, having been referred to the dermatologic evaluation of the pigmented lesion. The patient had previous history of excision of lesions in the face and left forearm ten years before, with clinical impression of skin cancer, without histological confirmation. The patient had previous diagnoses of systemic arterial hypertension, dyslipidemia and insomnia. He made continuous use of captopril, omeprazole and clonazepam. Also, he had previously undergone herniated disc surgery. He denied current or previous drug allergies, smoking habits, and alcoholism. He reported a history of ocular cancer in a first-degree cousin and denied a positive family history for skin cancer.

The clinical examination of the patient evidenced a hyperpigmented asymmetric macula, with various shades of brownish color, ranging from light to dark, with irregular borders, accompanying the left inferior palpebral rhyme (Figure 1). Dermoscopic examination revealed a thick pigmented network with eccentric pigmentation area in "blur" invading the follicular openings, as well as the presence of globules at the periphery of the lesion (Figure 2). The dermoscopic impression was of a cutaneous melanoma.

The patient was referred to the Oculoplastics department of the Ophthalmology Service for diagnostic surgical intervention. Since there was presence of trichiasis in the central region of the lower left eyelid, a total thickness resection of the central region of the left lower eyelid, in the shape of a pentagon, was carried out aiming at removing the area of poorly positioned eyelashes and obtaining material for histological analysis of the lesion. In the face of the anatomopathological diagnosis of lentigo maligna (Figure 3A), which was corroborated by the positive immunohistochemistry for Melan A (Figure 3B) and HMB-45, the safety margin was widened to 5mm, which corresponded to the total resection of the left lower eyelid. The reconstruction of the lower eyelid was performed with a Mustardé rotation flap. Considering aspects of the surgical treatment of the neoplasia,

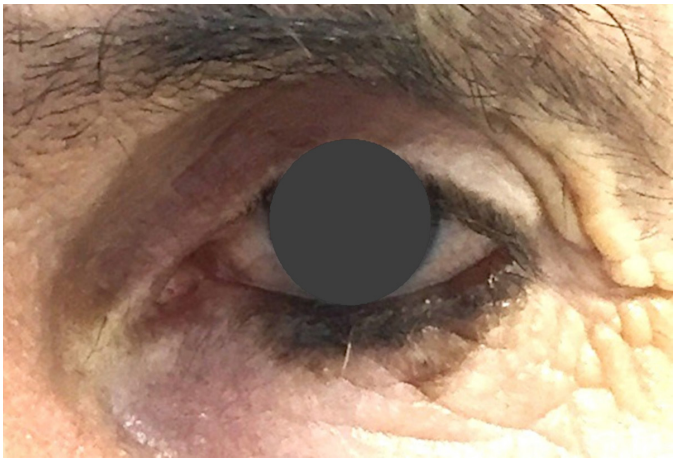


Figure 1: Infra-palpebral hyperpigmented spot

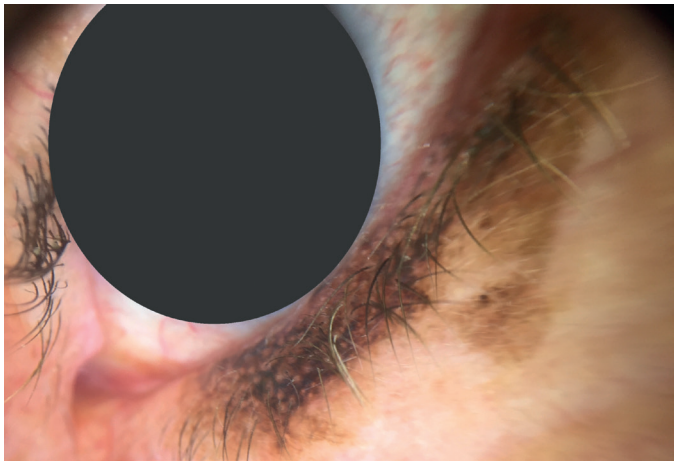
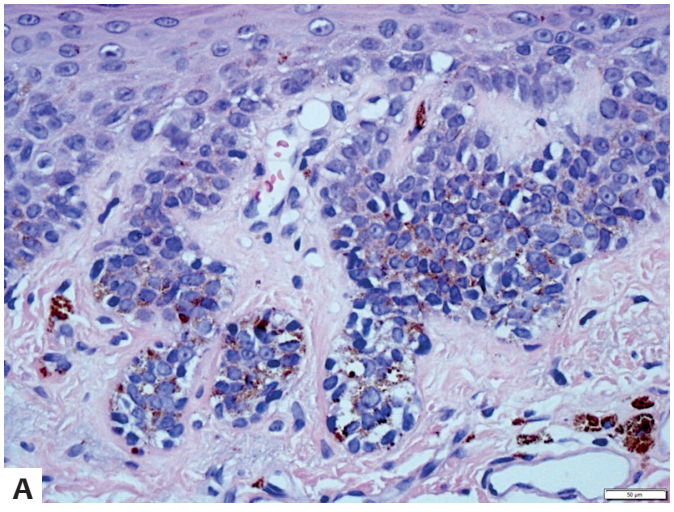


Figure 2: Dermoscopic appearance of the lesion



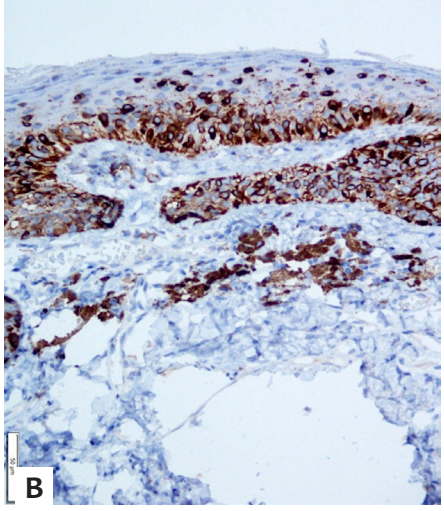


Figure 3:
A. Atypical intraepithelial melanocytes, characteristic of lentigo maligna (Hematoxylin & Eosin, 40x). **B.** Positive immunohistochemistry for Melan A (10x)

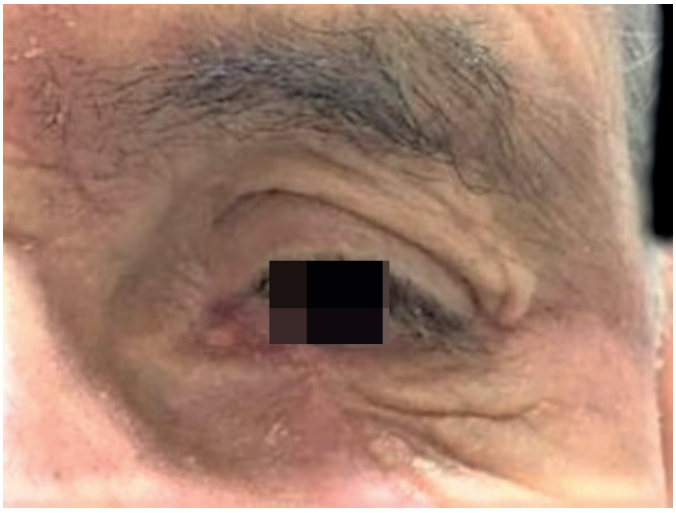


Figure 4: : Post-operatory left infrapalpebral appearance

the ocular apparatus' functionality, and facial aesthetics, the therapeutic outcome was outstanding (Figure 4).

DISCUSSION

Unlike in other body regions, margin expansion in periocular melanocytic lesions is challenging, since small amounts of resected tissue can lead to significant functional and aesthetic impairment. Often, it is necessary to limit the expansion of the margin to what is considered surgically acceptable in terms of resection and reconstruction. In the present case, a total thickness eyelid resection was chosen due to the involvement of the palpebral skin up until the mucocutaneous junction, even if there was no involvement of the palpebral conjunctiva.

Interestingly, there is no consensus as to the necessity and/or extent of expansion of safety margins in the conjunctival face when there is no compromise beyond mucocutaneous junction. Although surgical resection is the gold standard method, non-surgical options, such as the use of imiquimod, have been introduced with interesting outcomes in the treatment of *in situ* lesions of the periocular region.[3,5]

However, following the principle of oncologic surgical treatment for the management of primary cutaneous melanomas seems to grant primordial safety, with unprecedented cure rates. The observation of oncological principles must be associated to the concern about the functionality and aesthetics resulting from the intervention.[4]

CONCLUSION

For obtaining the best results from complex situations, specialized teams are crucial and should join forces in productive interaction.

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Case Reports

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Spitzoid melanoma simulating vascular lesion - Case report

Melanoma spitzoide simulando lesão vascular - Relato de caso

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ABSTRACT

Malignant melanoma is a melanocyte tumor responsible for more than 75% of skin cancer deaths. The rare variants of this pathology are responsible for 5% of the cases and may mimic other pathologies. We report the case of a patient with spitzoid melanoma and we discuss the dermoscopic, histopathological, and immunohistochemical findings, as well as the follow-up of this rare variant of melanoma.

Keywords: Melanoma; Nevus, epithelioid and spindle cell; Melanoma-specific antigens; Skin neoplasms; Dermoscopy

RESUMO

O melanoma maligno é um tumor de melanócitos responsável por mais de 75% dos óbitos por câncer de pele. As variantes raras desta patologia são responsáveis por 5% dos casos e podem mimetizar outras patologias. Relatamos caso de paciente com melanoma spitzoide e discutimos os achados dermatoscópicos, histopatológicos e estudo imuno-histoquímico, assim como o seguimento desta rara variante de melanoma.

Palavras-Chave: *Neoplasias cutâneas; Dermoscopia; Nevo de células epitelioides e fusiformes; Antígenos específicos de melanoma*

INTRODUCTION

Corresponding to less than 5% of all melanomas, rare histological variants may mimic other malignant tumors,[1,2] which often makes early diagnosis difficult.

The spitzoid variant of melanoma is rare and underdiagnosed due to its unusual clinical appearance and lack of pigment. Sometimes, definitive diagnosis can only be established after the onset of metastases.[3]

CASE REPORT

A 52-year-old female patient was referred to the care of the authors of the present article due to the presence of an asymptomatic violaceous lesion in her left thigh that had emerged two years before. The patient had Fitzpatrick IV phototype, and fibromyalgia and rheumatoid arthritis history, in addition to an intermittent and significant sun exposure without photoprotection throughout her life.

Dermatological examination revealed a well-defined erythematous papule with a violaceous center, measuring 1cm in its longest diameter (Figure 1). Dermoscopic analysis showed a central violaceous area devoid of structures, with white reticulated lines with erythematous interior and halo interspersed by red dots (Figure 2).

Diagnostic hypotheses of hemosiderotic dermatofibroma, thrombosed angiokeratoma and amelanotic melanoma were raised.

The authors of the present article chose to perform an excisional biopsy with pathological examination of the material, which suggested the presence of a spitzoid subtype of invasive malignant melanoma in its vertical growth phase, Clark level IV, Breslow thickness 1,1mm and 1 mitosis/mm2, without perineural or perivascular involvement. The presence of pagetoid ascension (Figure 3) and spindle cells nests (Figure 4) was key for

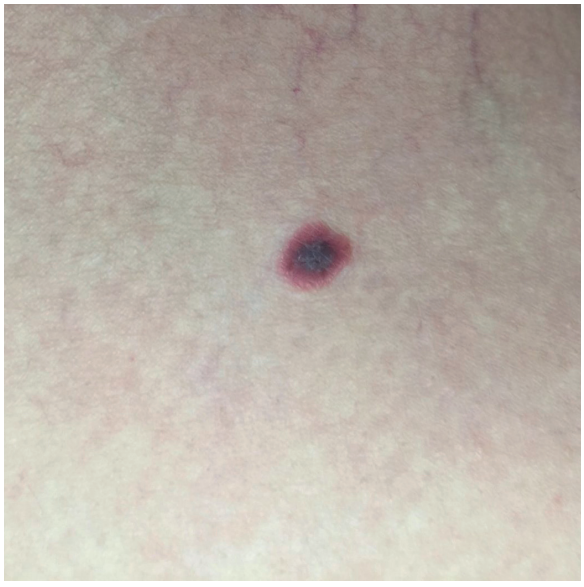

Figure 1: Erythematous papule and violaceous center in the left thigh

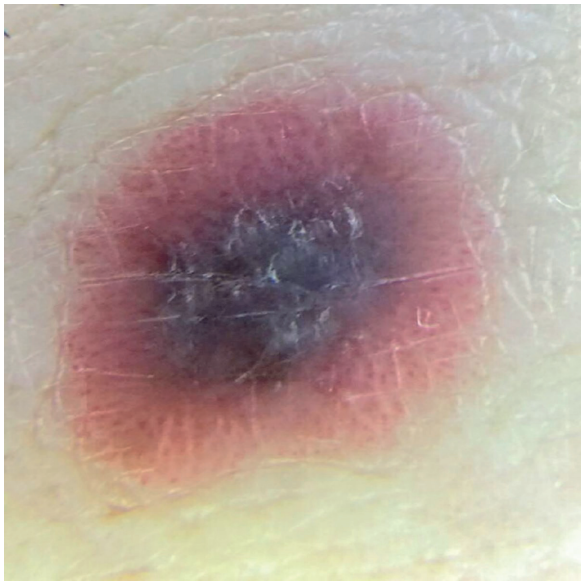

Figure 2: Dermoscopy: central violaceous area devoid of structures and erythematous halo, with cross-linked white lines throughout the lesion

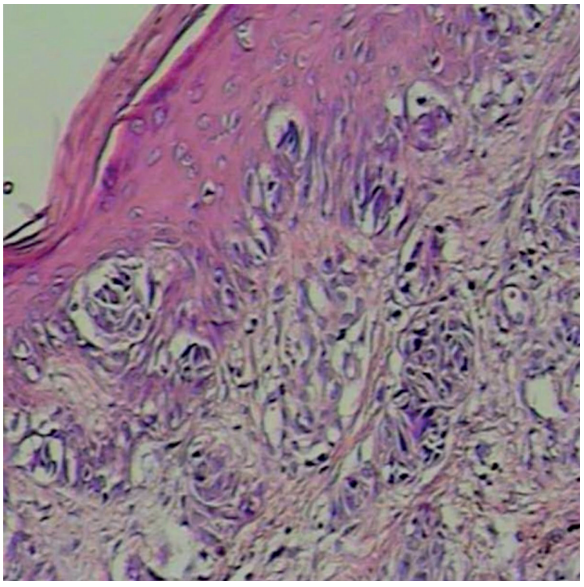

Figure 3: Anatomopathology: presence of nests of spindle cells

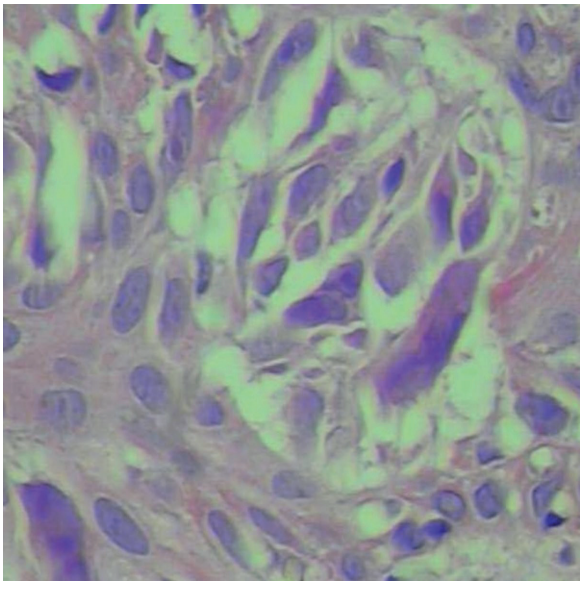

Figure 4: Cell atypias and spindle cells

Chart 1: Immunohistochemical study	
Researched antigens	**Results**
HMB-45	Positive in dermal and intraepidermal atypical melanocytes
KI-67	Positive in 2% of atypical melanocytes
MELAN-A	Positive in dermal and intraepidermal atypical melanocytes

this difficult diagnosis. The pathologist physician suggested the performance of an immunohistochemical study for diagnostic complementation. The results can be seen on chart 1.

After the surgical margins were widened (2cm) according to protocols recommended by the Melanoma Brazilian Group (GBM), the patient was referred to the oncological surgeon for sentinel lymph node biopsy, with negative outcome.

The patient is currently being periodically followed-up by Dermatology and Oncology teams.

DISCUSSION

Spitzoid melanoma occurs more frequently in the head and extremities, and shares many clinical and histopathological similarities with the Spitz nevus. [3], [4], [5] Papagiorgiou et al. consider that young age is an important diagnostic clue for the spitzoid subtype, a factor that has not been demonstrated in the present case report. [6]

Spitzoid melanomas often have the clinical appearance of amelanotic nodules, with crusts and ulceration, and have the following characteristics as differential diagnosis: hemangioma, pyogenic granuloma, xanthogranuloma and basal cell carcinoma.[4]

Although the clinical diagnosis is more difficult due to lack of pigment, the study by Semkova K et al. found no difference in mortality rates between melanoma and its spitzoid subtype.[7]

One of the greatest difficulties for the pathologist physician is the differentiation between Spitz nevus and spitzoid melanoma. Characteristics that corroborate the diagnosis of spitzoid melanoma include: size in excess of 1cm, ulceration, deep dermal penetration, asymmetry, lack of circumscription, absence of Kamino bodies, high degree of cytological atypia and of mitotic rate.[8, 9] Most of these criteria could be observed in the case described by the present paper.

In cases of atypical histopathologic characteristics, immunohistochemical analysis is crucial. In the present case, the authors investigated HMB-45, KI-67 and Melan-A. The Melan-A antigen peptide is one of the most studied, and is expressed in healthy melanocytes and melanomas. It is most commonly used in addition to the S-100 protein and the HMB-45 dye, for the immunohistochemical identification of malignant melanoma. [10] In face of this immunohistochemical profile, associated with the clinical-pathological findings, it is possible to determine whether the lesion is a spitzoid variant melanoma.

Although some studies have demonstrated the importance of the CD99 marker in the differentiation between Spitz nevus and spitzoid melanoma, this research was not used in the present case report. [11]

The histopathological diagnosis of the described case was established by an experienced dermatopathologist physician.

CONCLUSION

Identifying this rare melanoma subtype of atypical morphology is crucial due to the high mortality rate associated with the disease.

When anatomopathological analysis is not enough to reach clear diagnosis, immunohistochemical methods are an important tool for diagnostic assistance.

Long-term follow-up of the patient is instrumental for conducting studies on the prognosis associated with this atypical morphology.

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Yu´s flap for lip reconstruction

Retalho de Yu na reconstrução de defeitos labiais

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ABSTRACT

The reconstruction of defects involving up to 2/3 of the lip extension is challenging, considering the functional and aesthetic importance of this structure. There are several surgical techniques described for this purpose, highlighting the Abbé-Estlander flap, the Karapandzic flap, and the Bernard-Burrow-Webster flap. The choice of the best approach should consider the size and location of the defect as well as the comorbidities of the patient. The reverse Yu flap is an alternative to be considered in relation to other methods, allowing obtaining excellent cosmetic-functional results in general.

Keywords: Surgical flaps; Reconstruction; Lip

RESUMO

A reconstrução de defeitos que envolvam até 2/3 da extensão dos lábios é desafiadora, levando-se em consideração a importância funcional e estética desta estrutura. Existem diversas técnicas cirúrgicas descritas para esta finalidade, salientando-se o retalho de Abbe-Estlander, o retalho de Karapandzic e o retalho de Bernard-Burrow-Webster. A escolha da melhor abordagem deve levar em conta a dimensão e localização do defeito e as comorbidades do doente. O retalho de Yu constitui uma alternativa a ser considerada em relação a outros métodos, permitindo obter geralmente excelentes resultados cosmético-funcionais.

Palavras-chave: *Lábio; Reconstrução; Retalhos cirúrgicos*

INTRODUCTION

Considering the functional and aesthetic importance of the lips, many techniques have been described for the reconstruction of surgical defects in this region. The most relevant factors for the choice of the reconstructive approach are the labial defect's size and location, taking into consideration the limitations imposed by the patient's general state and comorbidities.

Yu *et al.* described an innovative flap in 1989[1] aimed at reconstructing surgical defects in the lower lip. This flap can also be used in a reverse way for reconstructing defects in the upper lip.

METHODS AND RESULTS

The Yu's flap is in fact the result of the combination of three local flaps:

a) a first sliding rhomboid flap that advances towards the labial defect, starting from the buccinator muscle area (its base located superiorly when the defect is in the upper lip, and vice-versa);

b) a second sliding rotation flap, roughly triangular in shape, used to cover the first flap donor area;

c) a jugal mucosa flap aimed at reconstructing the vermilion of the lip.

The surgical strategy can be summarized as follows[2] (Figures 1-3):

1. Curvilinear incision carried out along the nasolabial sulcus, extending by 1cm below the commissure.

2. Horizontal incision from the labial commissure up until to the previously performed labial incision. The length of this incision should be identical to that of the surgical defect's width when the Yu's flap is performed unilaterally. When performed bilaterally, the length of the incision should be half of the defect's width.

3. The first flap is raised in the subcutaneous plane observing the limits of the previous incisions, and advanced to the midline so as to cover the surgical defect. The second flap is subsequently dissected in a way that the skin and subcutaneous tissue of the nasolabial region rotates and advances to reconstruct the remaining defect of the first flap's donor zone. A discharge incision is carried out perpendicularly to the nasolabial groove in order to allow the rotation of the flap.

4. A third flap prepared from the jugal mucosa and roughly triangular in shape is raised in a submucosal plane aimed at reconstructing the vermilion of the lip.

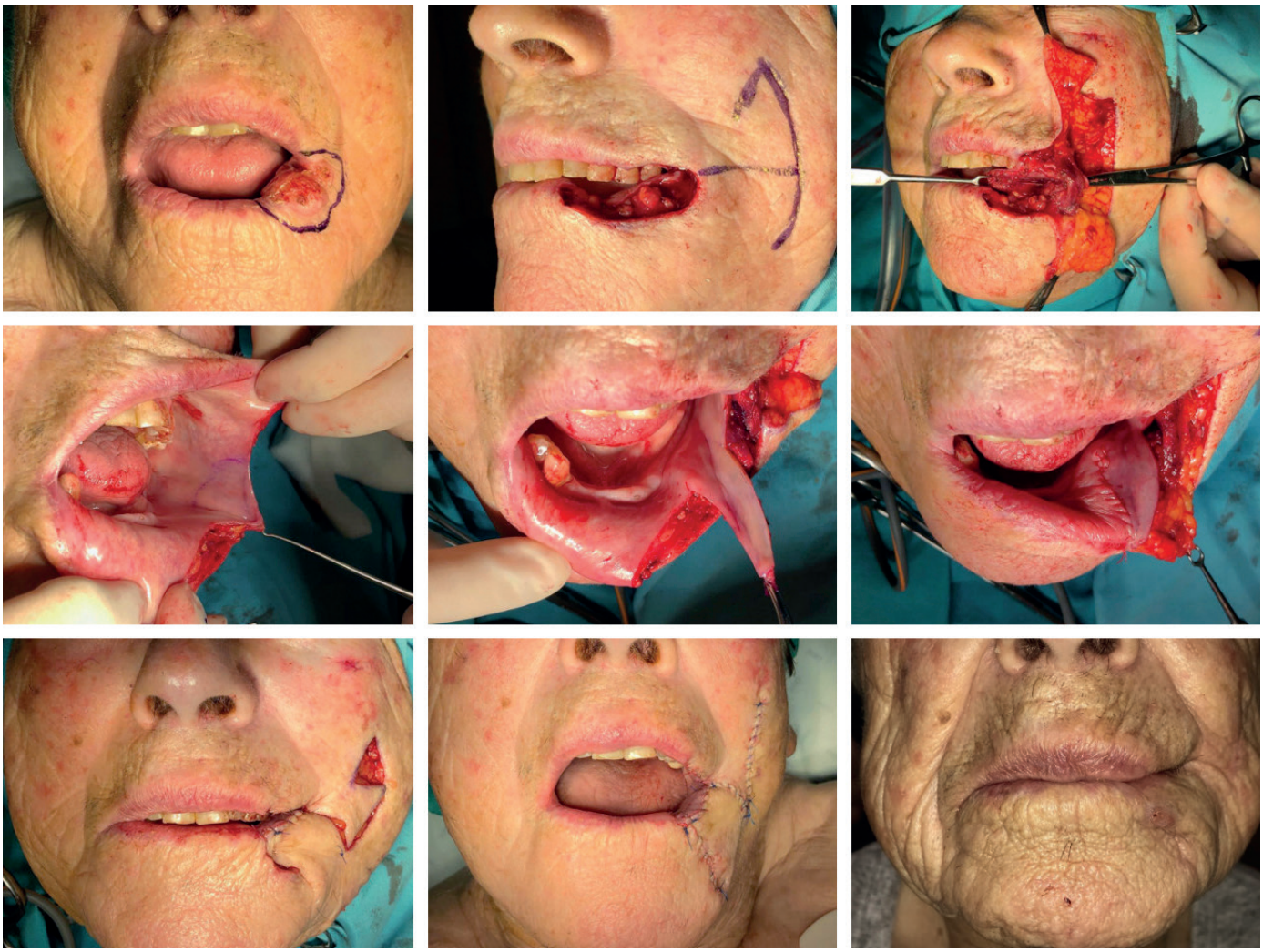

Figure 1: : Case 1 – SCC in the lower lip. Unilateral Yu's flap

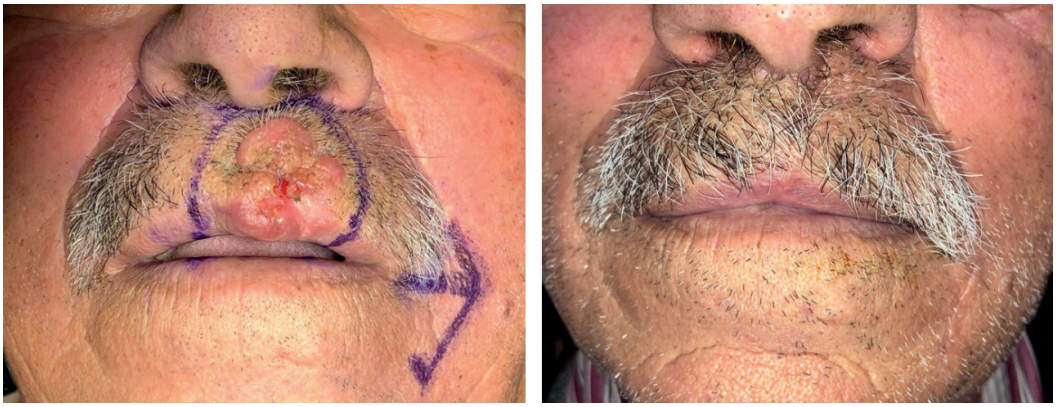

Figure 2:
Case 2 - SCC in the upper lip. Unilateral inverted Yu's flap

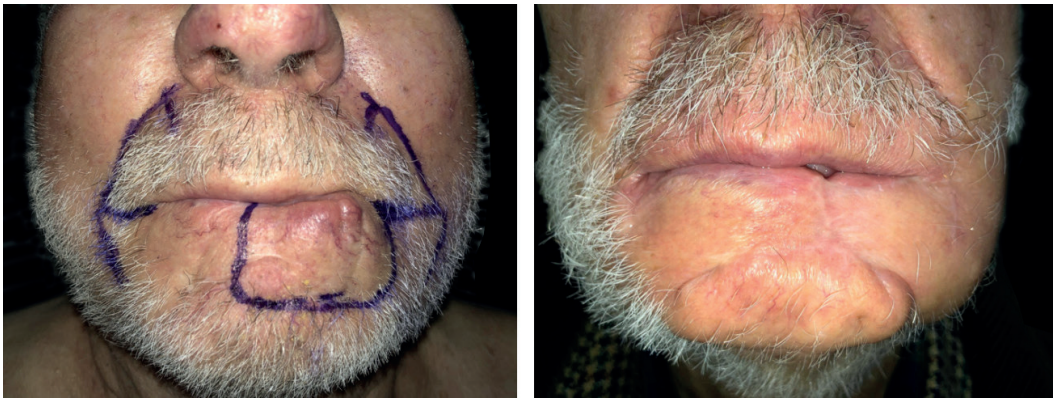

Figure 3:
Case 3 – SCC in the lower lip. Bilateral Yu's flap

The Yu's flap is a good option when the surgical defect covers more than 1/3 of the lip's length. Thus, when applied unilaterally, it can cover defects of up to 2/3 of the lip's extension. If performed bilaterally, it is able to repair defects covering 2/3 of the lip's length or even subtotal defects. [2, 3]

The authors of the present study applied this technique in the reconstruction of three extensive labial defects, all of which originated from radical excisions of squamous cell carcinomas.

Case 1: A 90-year-old woman bore a tumor with 2cm and erosive-crusted surface, located at the lower left 1/3 of the lower lip, that had emerged seven months before. Tumor resection resulted in a surgical defect covering more than 1/3 of the lip's extension. A left unilateral Yu's flap was implemented under left infraorbital and mental nerve block (Figure 1).

Case 2: A 82-year-old man bore a squamous cell carcinoma measuring 3cm on the longest axis, covering the middle third of the upper lip. Radical excision of the tumor led to a total thickness defect of the lip that reached almost half of its extension (Figure 2). An inverted unilateral Yu's flap was performed under bilateral infraorbital nerve block.

Case 3: An 88-year-old male patient bore a recurrent squamous cell carcinoma measuring roughly 3cm on the longest axis that covered the left inferior 2/3 of the lower lip. Its excision led to a total thickness defect covering between 1/2 and 2/3 of the lower lip's extension (Figure 3). Under general anesthesia, the patient underwent a bilateral Yu's flap.

There were no complications in any of the cases. The cosmetic-functional outcome was considered favorable in all cases, with preservation of oral continence and absence of microstomy.

DISCUSSION

The Yu's method is advantageous for allowing functional preservation of the lip by keeping the direction of the orbicularis muscle's fibers and preserving the oral continence. It preserves the location and shape of the labial commissure, maintaining asymmetry with the opposite side, even when performed unilaterally. This particularity is key for achieving a good aesthetic outcome. Owing to the fact it does not cause limitations for feeding and speech, it is a surgical procedure that allows a rapid return to normal life.

When compared to other flaps used for the same purpose – namely Karapandzic's[4, 5], Bernard-Burrow-Webster's [6, 7] and Abbe-Estlander's [8] – the Yu's flap has advantages and rewarding cosmetic outcomes.

The Karapandzic's flap is associated with higher microstomy rates with risk of distortion of the labial commissures.[9, 10] Despite not leading to microstomy, the Bernard-Burrow-Webster's flap usually results in some degree of incontinence of the oral sphincter, particularly at the commissures, and may also lead to a retraction of the lip relative to the expected position.[10, 11]

The main disadvantage of the Abbe-Estlander's flap is the need for two surgical times, in special when the surgical defect does not involve the commissure as well as risk of microstomy. [12]

It is important to note that, when used for reconstruction of subtotal and total defects, the Yu's flap may also cause microstomy. [13] Despite its greater surgical complexity, this flap carries a very low risk of injury to important structures. Nevertheless, it is necessary to preserve the fibers of the orbicularis oris muscle due to the risk of impairing oral continence in case it is destroyed.

By preserving the oral competence and symmetrical opening of the mouth, the Yu's flap guarantees good aesthetic outcomes in a single surgical time. If and when implemented, it implies a short hospital stay with a rapid return of the patient to his or her activities and daily routine.

CONCLUSION

The Yu's flap is an excellent alternative for the reconstruction of extensive defects of the lip, with a cosmetic-functional outcome that can be deemed advantageous over other procedures described for the same purpose.

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Surgical flaps for closing synchronous facial defects

Retalhos cirúrgicos para fechamento de defeitos faciais sincrônicos

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ABSTRACT

There are a few descriptions in the literature on surgical techniques for the closure of synchronous facial defects. We report three cases of patients with synchronous lesions on the face, which were reconstructed using the following types of flaps: Yin-Yang double--opposing rotation; Burrow's triangle advancement; and a combination of the dorsal nasal flap with the transposition flap. Reconstructions of synchronous facial defects represent challenges to dermatologic surgeons, especially when one of the lesions is located in the nose. In cases involving relatively small defects and not so far apart, there is the possibility of a single flap to close both defects.

Keywords: Surgical flaps; Mohs surgery; Carcinoma, basal cell

RESUMO

Poucas são as descrições da literatura sobre técnicas cirúrgicas para fechamento de defeitos sincrônicos na face. Relatamos três casos de pacientes com lesões sincrônicas na face, que foram reconstruídas utilizando-se os seguintes tipos de retalhos: dupla rotação Yin-Yang, avanço de dois triângulos de Burrow e combinação de retalho dorsal nasal associado a retalho de transposição. As reconstruções de defeitos sincrônicos na face representam desafios aos cirurgiões dermatológicos, especialmente quando uma das lesões se localiza no nariz. Nos casos que envolvem defeitos relativamente pequenos e não tão distantes entre si, há a possibilidade de se realizar um retalho único para fechamento de ambos os defeitos.

Palavras-Chave: *Retalhos cirúrgicos; Cirurgia de Mohs; Carcinoma basocelular*

INTRODUCTION

The most common body site for the occurrence of skin cancer is the face. Surgical treatment of these lesions requires an approach based on oncological principles associated to functional and aesthetic reconstructions. [1]

The literature brings together numerous reports of flaps being used to close single facial defects, however there are few descriptions of simultaneous closure of two lesions located near to each other. [1] The synchronous occurrence, excision and closure of facial lesions in the same surgical act represents a challenge to the dermatological surgeon.

CASE REPORT

Case 1 – A 76-year-old female patient, with absence of comorbidities, underwent Mohs micrographic surgery (MMC) for the excision of a superficial basal cell carcinoma (BCC) (initial size: 0.8cm x 0.6cm) in the right nasal wing and a well differentiated squamous cell carcinoma *in situ* (initial size: 0.4cmx 0.4cm) in the nasal dorsum. Only one MMC phase was required for complete resection of both tumors. The final defects measured 1.2cm x 1.2cm (nasal wing) and 0.6cm x 0.6cm (nasal dorsum) (Figures 1A and B). Due to the proximity of the lesions and the relatively small size of the defects, it was possible to use the untouched skin located between the lesions to perform a Yin-Yang double-rotation flap to close both defects (Figures 1B and C). On the seventh postoperative day, the aesthetic-functional outcome was very satisfactory, without any distortion of the nasal tip or wing (Figure 2).

Case 2 - A 53-year-old male patient, with absence of comorbidities, bore three asymptomatic lesions on the face for one year. The lesions were operated in the same surgical procedure using the MMC technique. In the glabella, he had a solid and micronodular BCC measuring 1.3cm x 0.9cm, which required only one phase for the complete resection. The final surgical defect of this lesion measured 2.2cm x 1.3cm and the closure was carried out directly (edge to edge).

Additionally, there was a solid BCC measuring 1.0cm x 1.0cm in the left lateral nasal wall, that was fully resected with two MCC phases (final defect: 1.9cm x 1.9cm). Close to this defect, in the left malar region, a micronodular BCC was also resected (initial size: 0.6cm x 0.5cm) in a single MMC phase (final defect: 1.4cm x 1.3cm). The untouched skin located between the defects allowed the implementation of a two-triangle Burrow's advancement flap (Figure 3).

Case 3 – A 70-year-old female patient, former rural worker, had three facial lesions that were resected by MMC (in a single phase). The first lesion was an adenoid BCC (0.6cm x 0.5cm) in the right supralabial region, whose final defect (1.4cm x 1.0cm) was closed edge to edge.

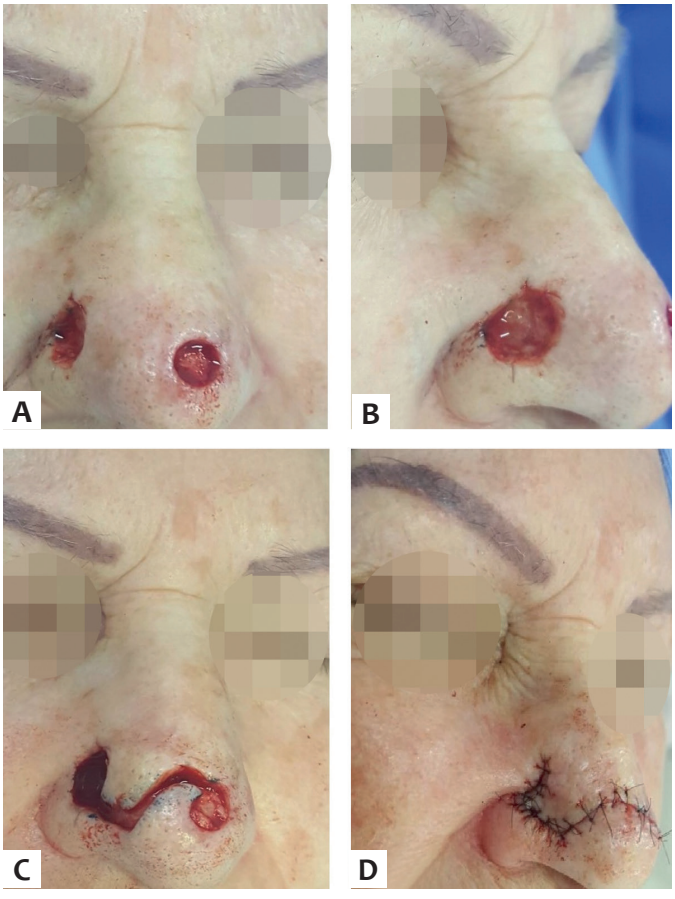

Figure 1: A - Synchronous surgical defects (right nasal wing and tip); **B -** Detail of the nasal tip lesion; **C -** Yin-Yang double-rotation flap marking; D - Immediate postoperative

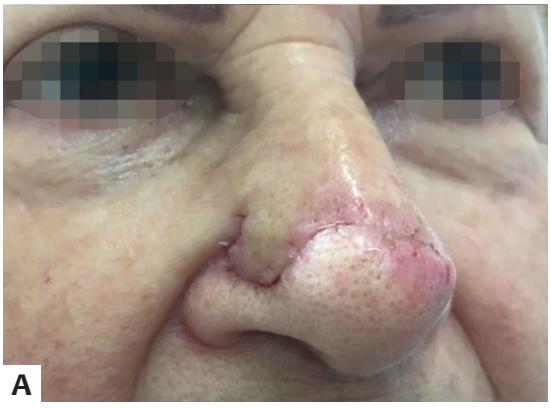

Figure 2: Seventh postoperative day, with a very satisfactory aesthetic-functional outcome

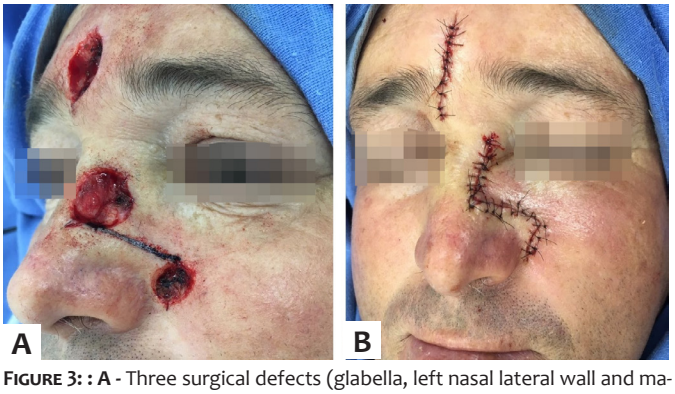

Figure 3: : A - Three surgical defects (glabella, left nasal lateral wall and malar). Note the drawing of the two-triangle advancement Burrow's flap between the nasal and malar defects. **B -** Immediate postoperative, with the glabella lesion closed edge to edge

The second lesion was a solid BCC (1.9cm x 1.3cm) in the nasal tip and dorsum, whose final surgical defect measured 2.4cm x 1.7cm. In the left nasal wing, another solid BCC (third lesion) could be observed (0.8cm x 0.6cm), whose surgical defect measured 1.2cm x 0.8cm. The size and location of the lesions did not allow the closure of both defects with a single flap. The authors of the present paper then chose to use a combination flap: a nasal dorsal flap aimed at closing the larger defect, and the untouched skin between the defects being used to transpose and close the nasal wing's defect (Figure 4). There were no distortions of the wing or nasal tip entailing from the implementation of the flaps.

DISCUSSION

In all three cases, at least one of the lesions was located in the nose, which is the facial site most frequently affected by skin cancer (25.5% of the cases) and also the one that implies greater difficulty for reconstruction. [3], [4] When surgical defects are double – and especially when at least one of them is located in the nose – great technical knowledge and a certain degree of creativity is required from the dermatologist physician. [2]

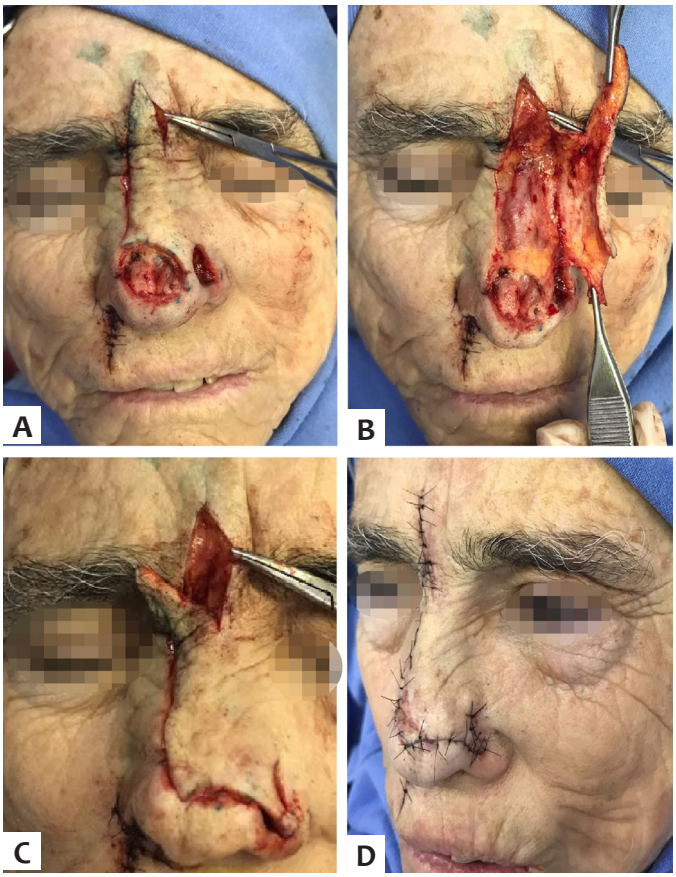

Figure 4: A: Surgical defect in the nasal tip / dorsum and left nasal wing, with the dorsal nasal flap having already been incised. **B -** Detachment of all the tissue that covers the nasal dorsum. **C -** Demonstration of final defect closure: the nasal dorsal flap covering the nasal tip/dorsum's defect and the transposition of the skin located between the two defects is capable of closing the nasal wing defect. **D -** Final surgical outcome

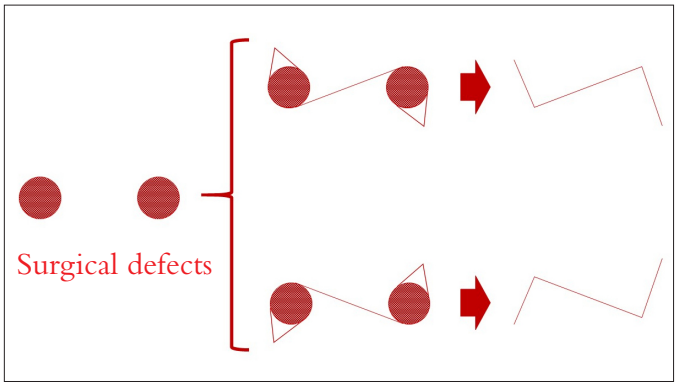

Figure 5: Drawing options for the two-triangles Burrow's advancement flap

In case 1, a double rotation flap was used for concomitant closure of the lesions. A curved line with the shape of an "S" is drawn tangentiating the two defects, with the double rotation movement being subsequently performed. Such a flap is also called Yin-Yang, in view of the similarity that flap's design has to the traditional Chinese symbol. [1]

In case 2, the closure was carried out using a two-triangle Burrow's advancement flap. The flap's preparation is relatively simple: a line is drawn tangentiating the defects in a way to unite them. Next, the compensation triangles (Burrow triangles) are drawn – one in each defect – opposite to the tangential incision site. In this manner, for each pair of defects there are two possibilities for drawing this flap (Figure 5). It is up to the dermatological surgeon to evaluate which option is most favorable, in view of the tissue's mobility, skin's tension lines and the patient's rhytids. [5], [6] Even though the resulting suture line is long, it is rather aesthetic (Z-shaped) and promotes a decrease in tension along the incision line. [5], [6]

In case 3, the occurrence of two large surgical defects in body sites with poor tissue redundancy did not allow primary closure or reconstruction using a single local flap. [7] An option to close the larger defect – on the nasal tip and dorsum – would be performing a cutaneous graft. Nevertheless, the use of a tissue with another color, the possible demarcation of the borders, and the natural convexity of the nasal tip would tend to lead to an unfavorable aesthetic result. The authors of the present paper then made a decision for performing two independent flaps: a dorsal nasal flap and a transposition flap using the untouched tissue located between the two surgical defects. This association allowed the closure of both defects and avoided the superior deviation of the nasal wing.

The nasal dorsum's flap – or Rieger's flap – involves the rotation and advancement motions of the skin from the proximal two thirds of the nose and glabella for the defect's closure in the distal third of the nose. [8] It is useful for rebuilding defects up to 2.5cm in diameter. It is considered a safe and of low morbidity flap, despite involving large portions of tissue. [9] Using this flap, it was possible to close the larger defect (nasal tip). The untouched skin located between the two defects was used as a transposition flap for the closure of the smaller defect.

CONCLUSION

Reconstructions of synchronic defects on the face in a single surgical time is challenging to dermatological surgeons, is special when one of the lesions is located in the nose. In cases involving relatively small defects that are located not so far apart from each other, it is possible to implement a single flap to close both defects.

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