

Intense pulsed light in photoaging: a clinical, histopathological and immunohistochemical evaluation *

Luz intensa pulsada no fotoenvelhecimento: avaliação clínica, histopatológica e imuno-histoquímica

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Abstract: BACKGROUND: Intense pulsed light has been used in the treatment of photoaging without a full understanding of its mechanism of action.

OBJECTIVE: To study the effect of intense pulsed light on photoaging and on the skin immune response by means of a clinical and histopathological study, evaluating Langerhans cells (CD1), expression of intercellular adhesion molecule, of CD4 and CD8 lymphocytes and quantification of collagen and elastic fibers.

METHODS: In 2006 a total of 26 patients, aged 40 to 65 years, with phototypes II to III (Fitzpatrick scale), were treated for photoaging using intense pulsed light in five sessions with a monthly interval. All the patients were subjected to histological and immunohistochemical evaluation 6 months after treatment.

RESULTS: At the end of the treatment clinical improvement was observed in 76.92% of cases. This improvement was associated to a significant increase of collagen (51.33%) and elastic (44.13%) fibers. Intense pulsed light treatment led to a reduction of CD4 lymphocytes and did not alter the amount of CD8 lymphocytes. It also led to a significant increase of small, nonectatic blood vessels, positive intercellular adhesion molecule.

CONCLUSION: Facial treatment with intense pulsed light promoted major clinical improvement that was confirmed by histological examination of the skin. This technique is a good treatment option for skin photoaging because it is non-ablative, safe and effective.

Keywords: Collagen; Elastic tissue; Lasers; Skin aging; Rejuvenation

Resumo: FUNDAMENTOS: A luz intensa pulsada tem sido muito utilizada no tratamento do fotoenvelhecimento sem completo conhecimento de seu mecanismo de ação.

OBJETIVO: Estudar a ação da luz intensa pulsada no fotoenvelhecimento e na resposta imunológica cutânea por meio de estudo clínico, histopatológico, avaliando células de Langerhans (CD1), expressão da molécula de adesão intercelular, de linfócitos CD4 e CD8 e quantificação de colágeno e fibras elásticas.

MÉTODOS: Um total de 26 pacientes, com idades entre 40 e 65 anos, com fototipos II a III de Fitzpatrick, foram tratadas do fotoenvelhecimento usando LIP em 5 sessões, com intervalo mensal, durante o ano de 2006. Todas as pacientes foram submetidas à avaliação histológica e imuno-histoquímica 6 meses após o tratamento.

RESULTADOS: Ao término do tratamento, houve melhora clínica em 76,92% dos casos, estando relacionada ao aumento significante de fibras colágenas (51,33%) e elásticas (44,13%). O tratamento com luz intensa pulsada promoveu redução de linfócitos CD4 e não alterou a intensidade de linfócitos CD8. Além disso, promoveu aumento significante de pequenos vasos sanguíneos, não ectásicos, molécula de adesão intercelular positivos (não consegui entender se o procedimento promoveu aumento de vasos e de molécula ou se houve um aumento significante de molécula. Acho que falta algo antes desta expressão grifada em vermelho).

CONCLUSÃO: O tratamento facial com luz intensa pulsada promoveu intensa melhora clínica que foi comprovada pelo estudo histopatológico da pele, constituindo boa opção de tratamento para o fotoenvelhecimento cutâneo, por ser técnica não-ablativa, segura e eficaz.

Palavras-chave: Colágeno; Envelhecimento da pele; Lasers; Rejuvenescimento; Tecido elástico

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INTRODUCTION

Intrinsic or chronological aging is genetically determined, and extrinsic aging, also called photoaging, occurs by exposure to ultraviolet radiation.¹ With regard to the histological phenomena of aging, there are differences between intrinsic and extrinsic aging. In extrinsic aging, the epidermis shows hyperkeratosis, melanocytes are more numerous and there is flattening of the dermoepidermal junction. In the dermis, there is a wide band of eosinophilic material, called Grenz zone, an accumulation of elastic fibers, forming amorphous masses (elastotic material), fibroblasts are reduced in number and collagen fibers are thin.²

Although Intense Pulsed Light (IPL), developed by Goldberg, is not a laser, it is a non-coherent light that covers a large wavelength to be absorbed by the desired chromophore, as it directs a specific light beam to the target, through the use of cutting filters, exposure time of the light pulses and interval between them.³

Non-ablative photorejuvenation with IPL works by causing reversible thermal damage to collagen due to light penetration into the dermis and direct heating of these structures, sparing the epidermis. Therefore, contraction of collagen fibers and fiber remodeling are achieved after the inflammatory period.

PATIENTS AND METHODS

The study group consisted of 26 women, aged 40 to 65 years (mean age 51.7 years). The patients had skin phototypes II and III (Fitzpatrick Scale) and showed skin photoaging of the face, grade III (Glogau scale).^{4,5} Procedures were initiated after a detailed explanation of the research study and after the patients signed the free and informed consent. Exclusion criteria included patients previously submitted to any cosmetic procedure in the face, including treatment with IPL or Laser, tanned skin and/or phototypes V and VI (Fitzpatrick Scale), those with diseases aggravated by light, patients taking photosensitizing drugs, patients with a history of keloid or hypertrophic scarring, inability to use sun protection during treatment, active skin diseases in the face or refusal to sign the consent form.⁴

In this study patients underwent facial treatment with IPL and we conducted a pre- and post-treatment histopathological evaluation. For this, the following routines were adopted: 1 - thirty days before the start of IPL therapy, all the patients prepared their facial skin with retinoic acid 0.025% combined with hydroquinone 4% at night; 2 - after the start of treatment, patients were instructed to only use SPF 30 sunscreen on the face during the day and avoid direct sunlight; 3 - patients underwent photographic docu-

mentation of their face before and 6 months after initiation of treatment in three different positions: anterior, right and left lateral; 4 - skin biopsies were performed before and after treatment; 5 - facial treatment with 5 IPL sessions; 6 - histopathological study of the biopsies.

Skin biopsies were performed in the right preauricular region in an area affected by photoaging, using disposable "punch" n° 4 after infiltrative anesthesia with lidocaine 2% without vasoconstrictor. The suture was made with mononylon 6.0 and removed at day 5.

The patients were submitted to five sessions, with a monthly interval, with ILP equipment, Record 618, Israel. This equipment has broadband technology-GEM (Geometrical Energy Management), emits light at wavelengths between 420 and 1100 nm, pulse duration of 10 ms, single pulse, energy between 10 and 22 J/cm², program from 0 to 12 and air cooling. It has a tip of 1.5 x 5.0 cm. In this study we chose an energy of 20 J/cm² (program 10) for all the patients, which is the most suitable for phototypes II and III (Fitzpatrick Scale). A cooled transparent gel was used, forming a thin layer on the skin for better coupling of the tip to the skin and also to protect the epidermis.

At each session, IPL was applied to the entire face. Discharges were made adjacent to each other, and three applications were done. In the nasolabial fold region, glabellar region, perioral and periorbicular regions, two subsequent discharges were made. Protective goggles were used by patients and by the researcher during the procedure. For relief of redness and burning due to the procedure, patients were instructed to use cold compresses.

The evaluation of side effects (erythema, edema, burning and crusting) was conducted immediately after the completion of each session and in every return visit.

Clinical evaluation consisted of analysis of clinical photographs before and after treatment of each patient by three specialists in dermatology. Improvement was classified as slight, moderate and great in relation to texture, skin lightning and improvement of fine wrinkles of the face.

The patients were evaluated in relation to the degree of satisfaction with scores ranging from 0 to 10.

Histopathological evaluation was performed by histomorphometry with the aid of a Kontron 300 (Zeiss) image analyzer. Skin biopsies were immersed in 10% formalin solution in a phosphate buffer for 24 hours and were then submitted to histological routine. 3µm-thick cuts were obtained from the paraffin blocks and stained with Picrosirius for collagen fibers and Weigert-oxone for elastic fibers. The cuts under-

went immunohistochemical reactions for CD4 and CD8, ICAM-1 for blood vessels and CD1 for Langerhans cells. The quantification of collagen and elastic fibers and vessels was done by area fraction (%). The evaluation of Langerhans cells was obtained by the number of CD1 positive cells per area of epidermis (μm^2). Positive CD4 and CD8 cells in the dermis were evaluated by a semiquantitative method.

Data were analyzed by descriptive statistics: mean, standard deviation, minimum and maximum value and median. To assess whether treatment made a difference, we compared pre- and post-treatment data. The values were subjected to normality test. When the normality test was significant, we used the statistical test of analysis of variance. When values did not follow a normal distribution, we used the Kruskal-Wallis nonparametric statistical test. Statistical tests were performed using the SigmaStat (JandelScientific, CA, USA) software, with significance level of $p < 0.05$.

RESULTS

Clinical improvement of the skin after 6 months of treatment was moderate to great in 76.92% of the cases. ($p < 0.05$) (Figure 1).

Histopathological evaluation revealed marked and significant increase of 51.33% ($p < 0.05$) of collagen fibers and 44.13% of elastic fibers ($p < 0.05$) (Figures 2 and 3). We also observed that these newly formed collagen and elastic fibers had a uniform distribution following a parallel axis to the surface of the epidermis, involving the superficial, mid and deep reticular dermis.

The quantification of CD1+ Langerhans cells at baseline was 896.440 cells/mm² and 725.900 cells/mm² after treatment ($p = 0.083$), showing no significant difference.

Evaluation of CD8 lymphocytes showed no dif-

ference ($p = 0,123$) between pre-(1,0) and post-treatment (1,0), whereas in relation to CD4 lymphocytes a reduction of intensity (3,0) was observed after treatment, as compared to pre-treatment (2,5), and this difference was significant ($p = 0.5$).

There was a significant increase in area fraction of small blood vessels in the dermis, nonectatic, expressed by ICAM-1 positive, 6 months after initiation of treatment, ranging from 0.527% to 0.924% ($p < 0.05$) (Figure 4).

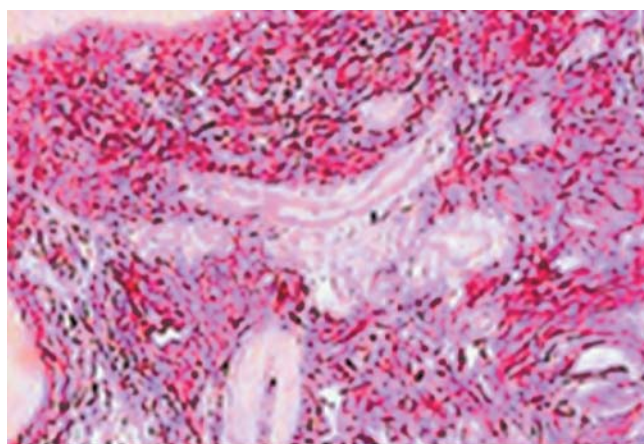
DISCUSSION

Non-ablative photorejuvenation is a method that has been extensively studied seeking the reversal of skin aging, through the use of IPL, to cause dermal damage without ablation of the epidermis. The dermis reacts to the aggression by increasing the production of collagen and reabsorbing elastotic material.⁶ Explanations for the synthesis of new collagen include the absorption of light by blood, which increases the temperature around the vessels, transferring thermal damage to surrounding tissue and causing the release of inflammatory mediators that induce the healing process. Energy would also directly stimulate fibroblasts to produce more collagen.⁷

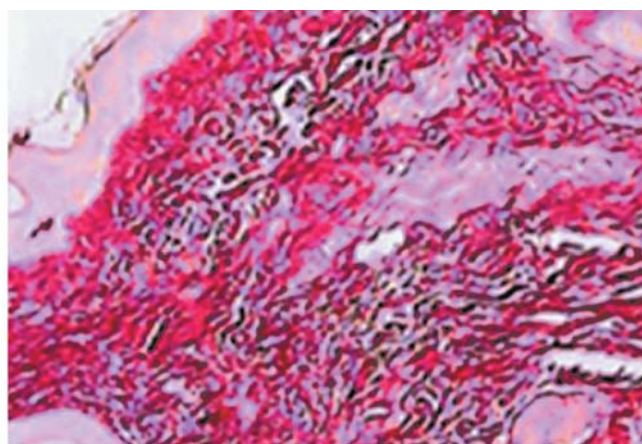
Patients in this study showed an improvement in flaccid skin related to the increase of collagen in the deep reticular dermis, which promoted a "skin tightening" effect in these patients after treatment.

These results were also obtained in studies that used different types of laser for non-ablative photorejuvenation to treat aging skin. These studies showed collagen synthesis and improved solar elastosis at different levels on histopathological evaluation.^{3, 6, 7, 8, 9, 10}

Side effects such as edema and erythema of photoaged skin, observed immediately after application of IPL, are due to inflammation of the skin, cau-

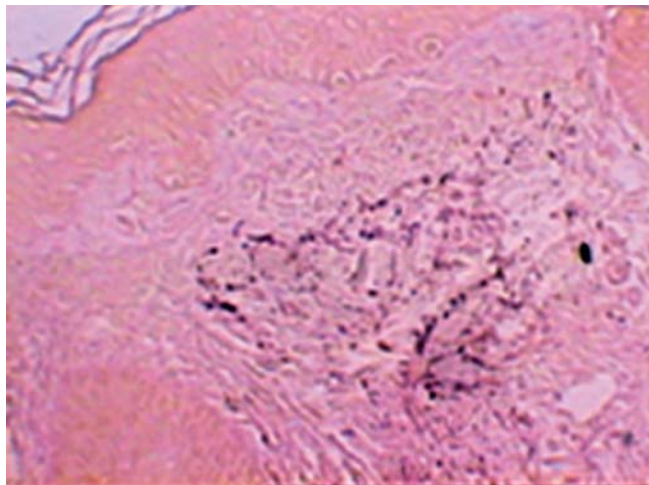


pre-treatment

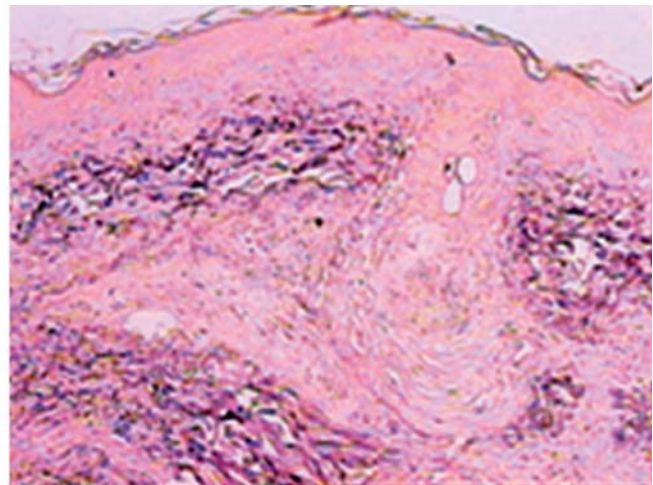


After 6 months

FIGURE 1: Histopathology revealed marked and significant increase of 51.33% ($p < 0.05$) of collagen fibers

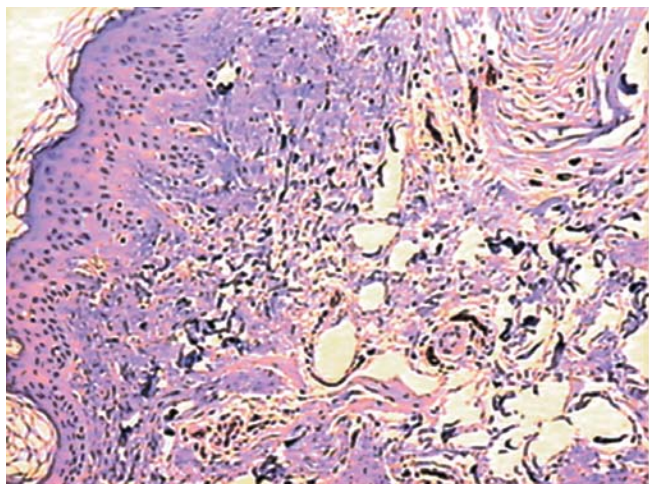


pre-treatment

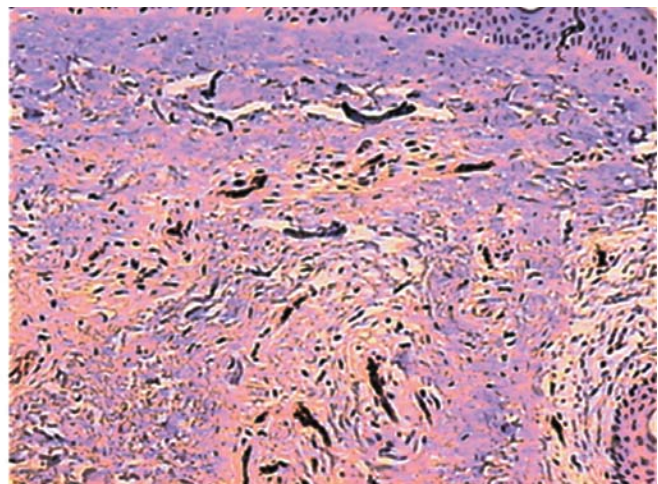


After 6 months

FIGURE 2: Histopathology revealed a significant increase of 44.13% of elastic fibers ($p < 0.05$)



pre-treatment



After 6 months

FIGURE 3: A significant increase in area fraction of small blood vessels in the dermis, nonectatic, expressed by ICAM-1 positive, 6 months after initiation of treatment, ranging from 0.527% to 0.924% ($p < 0.05$)

sed by the interaction of light with tissue, which releases heat.¹¹ These effects lasted from 24 to 72 hours and completely disappeared. In addition to edema and erythema, the possibility of blistering 24-36 hours after the session, with subsequent formation of crusts that disappear within 7 to 14 days, has been reported in the literature.¹¹ In the present study, 22% of the subjects had crusts on the face, which appeared 24 hours after the session and lasted about a week. Since their duration was shorter than the turnover time of the epidermis (four weeks), they were probably caused by superficial burning.¹²

Fournier states that in all processes of remodeling or “resurfacing”, it is expected that the Grenz zone thickens by increased collagen deposition, with

reorganization into a parallel arrangement with compact fibrils. He argues, however, that it takes months for this process to occur after the procedure.⁹

Sadick, in a study of non-ablative photorejuvenation, states that the results are delayed. Pigmentation, vascular and sebaceous changes are noted in 3 to 6 months, and reduction of wrinkles is observed after 12 to 18 months.¹³

Feng and Zhao declared that the mechanism of action of IPL for rejuvenation can be explained by increased activity of fibroblasts, fibroblast hyperplasia and rearrangement of collagen and elastin within the stroma.¹⁴

Li *et al.* evaluated the efficacy and safety of IPL in the treatment of facial photoaging in Asians; 89.5%

of the patients rated their overall improvement as excellent or good. Adverse effects were limited to mild pain and transient erythema.¹⁵

IPL treatment did not promote change in skin immunity in relation to CD1, CD4 and CD8. These findings have not been described in the literature. Salgado studied the ablative technique using carbon dioxide laser and found that the amount of CD4 and CD8 lymphocytes was the same, before and after 15 and 90 days of treatment.¹⁶

This study is the first to show an increase of ICAM-1 in the dermis 6 months after the start of treatment. This increase was statistically significant, showing an increase of blood capillaries in the dermis. It

is known that ICAM is a membrane glycoprotein of intercellular adhesion, and is associated with leukocyte adhesion to vascular endothelium. This change may be associated with inflammation, since ICAM-glycoprotein is produced when there is invasion of the vessel wall by leukocytes, or simply be evidence of neovascularization by reperfusion of pre-existing vessels.^{17,18}

CONCLUSION

IPL is a good treatment option for cutaneous photoaging. It is a non-ablative, safe and effective technique, since the clinical improvement observed by patients is supported by histological analysis. □

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