

The use of CO₂ laser in vulvar lichen sclerosis treatment — molecular evidence

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ABSTRACT

Objectives: Vulvar lichen sclerosis is chronic and difficult to treat disorder, which often is recurrent and leads to multiple complications. The limited efficacy of pharmacologic treatment directed the search for new therapies including use of CO₂ laser. The aim of the study was to provide rationale for the use of CO₂ laser in treatment of vulvar lichen sclerosis.

Material and methods: Two patients with diagnosed LS were recruited for the study. Vulvar biopsies were taken before CO₂ laser application and then 3 months later. We assessed the following genes: COL1A, COL1A2, COL3A1, COL5A1, COL5A2, COL5A3, ELN, ECM1, HSPA1A, HSPA1B, SERPINH1, TLL1, TP53, BMP1. We also assessed patients' quality of life by VuAS scale before and after treatment.

Results: In both patients we observed decreased clinical symptoms observed by an experienced gynecologist as well as significant changes in gene expression before and after laser treatment.

Conclusions: Treatment with CO₂ laser can be alternatively considered in patients with vulvar lichen sclerosis.

Keywords: vulvar lichen sclerosis; CO₂ laser; heat shock proteins; collagen; COL1A1; HSPA1A; HSPA1B

Ginekologia Polska 2023; 94, 11: 903–906

INTRODUCTION

Vulvar lichen sclerosis is chronic and recurrent inflammatory dermatitis which can lead to vulvar, perineal and perianal scarring and sexual dysfunction. It more often affects women than men and primarily postmenopausal women, however it is found in women of any age from young girls to old women [1]. Physical examination reveals ivory white atrophic plaques with a waxy texture or epidermal wrinkling ("cigarette paper" appearance), depigmentation or hyperpigmentation, ecchymoses, resorption of the labia, narrowing of the introitus, and distortion of the vulvar architecture [2]. The most common symptoms are itching, pain, skin fissures and painful sexual intercourse. The etiology of the disease remains unknown, and the following factors seem to contribute partially: autoimmune mechanism, genetic predisposition, connection with viral infections, trauma, prolonged irritation and hormonal changes [3]. The current gold standard of treatment includes local steroids (e.g., clobetasol propionate, mometasone furoate) which help alleviate symptoms and decrease inflammation [4]. The pharmacologic treatment is effective in majority of patients, but with time may lead to skin atrophy or increasing skin infections. However, some patients may present with side effects or lack of treatment acceptance and in those we offer the use of CO₂ laser [5]. In an Australian study, five women

with a severe, resistant to standard treatment form of hyperkeratotic lichen sclerosis were treated with a fractional CO₂ laser [5]. Four of them showed the remission of symptoms and further, the treatment was limited only to local medication with sufficient response. CO₂ laser has a wave of 10600 nm which is easily absorbed by water in tissue and allows for superficial effect of microablation in soft tissue. The pulse beam protects tissue from potential damage resulting from overheating. The laser beam is delivered in fractions, creating small, alternating dots both in treated and untreated tissue [6].

Objectives

To assess the changes in the expression of collagen and elastin genes as well as heat shock protein and p53 genes over time in the vulvar lichen sclerosis tissue after CO₂ laser application.

MATERIAL AND METHODS

Before the CO₂ laser was applied, each patient was examined vaginally to exclude active infection, presence of unusual skin marks and other potentially neoplastic lesions. The area of procedure included labia majora, labia minora, vaginal introitus, clitoral hood, perineum and perianal region. The skin was anesthetized using

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Received: 2.03.2023 Accepted: 8.03.2023 Early publication date: 20.04.2023

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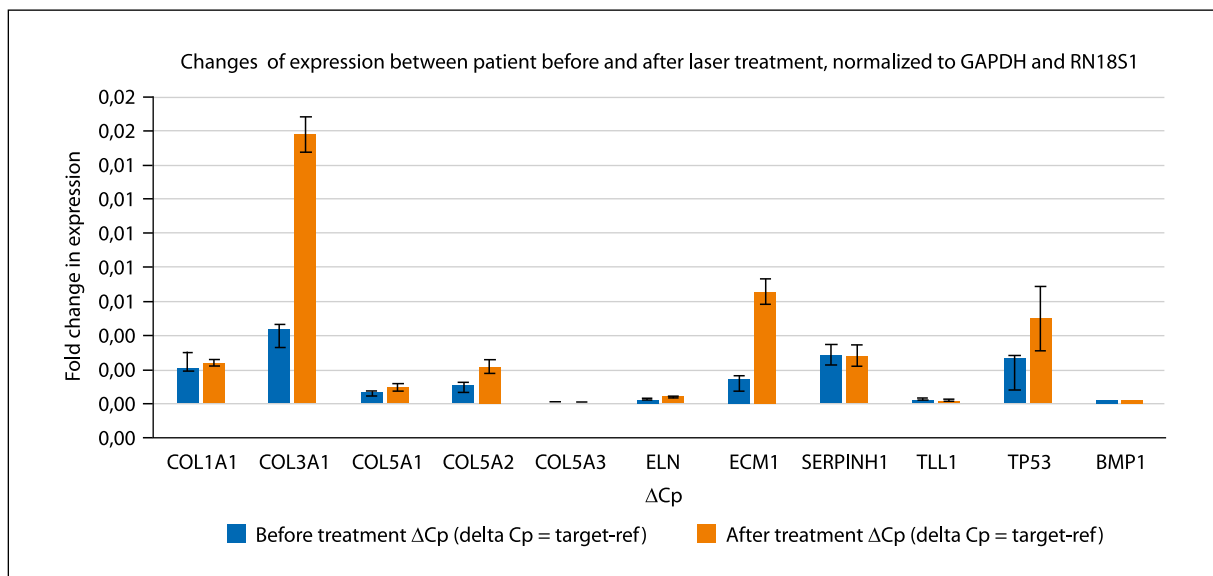


Figure 1. Changes in gene expression before and after treatment in patient A

cream with lidocaine which was applied 15–30 min before procedure. After procedure, if the vaginal or perineal discomfort was experienced, patient was advised to apply cooling pads and pain medications *e.g.*, paracetamol. Patients were advised to avoid sexual contacts for 7 days after procedure.

On the day of procedure, before the laser application, vulvar samples were taken bilaterally from abnormal tissue. Vulvar samples were sent for standard pathological evaluation and gene expression assessment. Tissue for the latter was put into a tube with RNAlater, and further molecular processing was done using RNase MiniKit (Qiagen, Hilden, Germany) with additional purification with DNase on channel, and further using RealTime PCR with 96–16 panel (Roche, Basel, Switzerland). The molecular panel included the following genes: COL1A, COL1A2, COL3A1, COL5A1, COL5A2, COL5A3, ELN, ECM1, HSPA1A, HSPA1B, SERPINH1, TLL1, TP53, BMP1.

After the vulvar samples were taken, the CO₂ laser was applied to area described above. Three months after the procedure, patients were followed up by the same experienced gynecologist (AO) who performed the clinical examination and simultaneously, patients completed the VuAS (Vulvar Assessment Scale) [7]. VuAS included four questions which allowed to assess the intensity of dryness, painfulness, feeling of itching with daily activities (question 1–3), and the increase of the intensity of these symptoms during sexual intercourse (question 4). During the follow up visit, the vulvar samples were taken from the similar locations as previously and processed in the same manner as at the study entry.

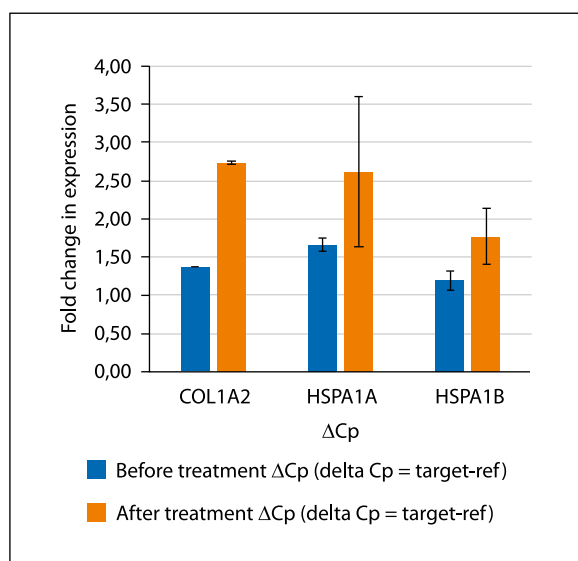


Figure 2. Statistically significant changes in COL1A2, HSPA1A and HSPA1b expression after CO₂ laser treatment in patient A

RESULTS

In both patients, we observed decreased clinical symptoms assessed by an experienced gynecologist (AO) and noted based on VuAS scale. Patient A showed moderate decrease of symptoms, while in patient B we noted full remission of symptoms 1–3, with persistent symptoms related to question 4 at the same level as before the CO₂ laser treatment.

We demonstrate changes between the gene expression before and after treatment with CO₂ laser. Figures 1 and 2 shows results in patient A, while Figures 3 and 4 in patient B.

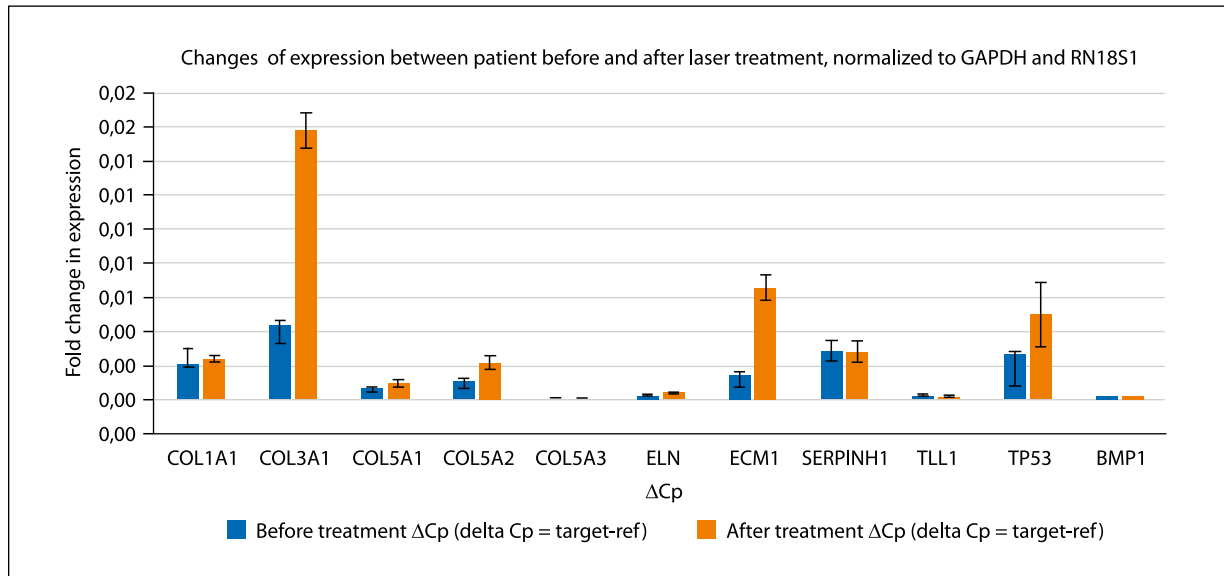


Figure 3. Changes in gene expression before and after treatment in patient B

DISCUSSION

Our study goal was to analyze changes in gene expression (including collagen, elastin, heat shock protein, p53) in two patients undergoing CO₂ laser treatment for *vulvar lichen sclerosis*. It is the first study to note statistically important changes in COL1A2 expression and heat shock proteins (HSPA1A i HSPA1B).

The previous study described the decrease in the serum level of collagen I in patients with burn scars treated with laser [8]. However, the main difference between our study and the previous one is that the former one assessed the level of protein in the serum- not the gene expression in the local tissue and thus may replicate the systemic response to treatment rather than a local one. The increase in the HSPA1A gene activity accounts for a normal skin cells response to thermal effect of laser. Previously, a mouse model showed that there is an increase in activity of HSPA1A after laser application, which is a protective process done by skin cells in response to cytotoxic thermal effect of laser [9]. On the other hand, the increase in HSPA1B expression was described in patients with skin squamous cell managed with a combination of chemotherapy and laser. So, our findings are supported by the previous research done both in animals and humans [10].

Additionally, our study adds clinical data to results of treatment with CO₂ laser in women with *vulvar lichen sclerosis*, formerly shown by Baggish [11]. In that study, 27 women with symptomatic *vulvar lichen sclerosis* underwent CO₂ laser treatment. 24 out of 27 claimed a decrease in itching, pain and vulvar discomfort. 26 out of 27 women had an improved skin color and elasticity. Our patients showed improvement in VuAS scale as well in clinical examination.

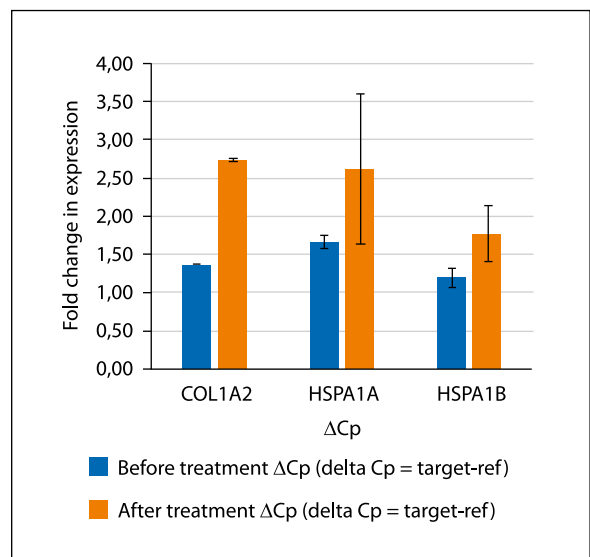


Figure 4. Statistically significant changes in COL1A2, HSPA1A and HSPA1b expression after CO₂ laser treatment in patient B

CONCLUSIONS

CO₂ laser contributes to significant changes in gene expression in *vulvar lichen sclerosis* tissue. It helps alleviate symptoms in women suffering from the disease. We suggest considering laser CO₂ treatment in women with this disease.

Article information and declarations

Funding

Medical University of Silesia fund.

Conflict of interest

All authors declare no conflict of interest.

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