



Vulvar lichen sclerosis: A new regenerative approach through fat grafting



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HIGHLIGHTS

- Lichen Sclerosis (LS) often leads to anatomical impairment prejudicing life quality.
- Vulvar Fat Graft is a completion therapy of LS, thanks to its regenerative power.
- Fat Grafting in Vulvar LS can improve anatomical impairments and sexual activity

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ABSTRACT

Objective. The aim of this study was to evaluate the efficacy of fat grafting in the treatment of severe vulvar lichen sclerosis (LS). Our primary outcome was to assess the improvement of mucocutaneous trophism, the resolution/reduction of symptoms, and the histological features of the vulvar skin after treatment. The secondary outcome was to evaluate the improvement in life quality, and in resumption and quality of sexual life.

Methods. Between 2011 and 2014, 36 patients were offered fat grafting to treat LS. Inclusion criteria were age between 25 and 80 years, histopathologic diagnosis of LS, good health, failure of previous first line treatments.

Results. 34 out of 36 patients (94%) showed a better vulvar trophism of the skin and mucosae; 27 (75%) had an improvement in caliber and elasticity of the vaginal introitus; clitoris burying degree was reduced in 18 patients (50%), 30 (83%) reported an increased volume of labia majora and minora, 34 (94%) had a complete disappearance of scratching lesions, and 28 (78%) showed a remission of white lesions. Eventually 34 patients (95%) stopped using topical corticosteroids routinely. The improvement in life quality was significant for both DLQI ($p < 0001$) and FSFI ($p < 0001$).

Conclusions. Fat grafting may have a role as a support and completion treatment in selected cases of women with vulvar LS who do not respond to first line therapy or in severe cases where the anatomical impairment does not allow a regular sexual function and a good quality of life.

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1. Introduction

First described in 1887, lichen sclerosis (LS) is a chronic, inflammatory, mucocutaneous disorder of genital and extragenital skin with a predilection for the anogenital region. Only 6% of LS involves isolated extragenital areas.

LS etiology is still uncertain, probably multifactorial, but there is an increasing evidence that autoimmune mechanism plays an important pathogenetic role, taking into consideration the strong association with many autoimmune disorders in 21–28% of cases [1–4].

Although many authors agree that the prevalence of LS is still under-diagnosed and difficult to be evaluated, the estimated incidence ranges between 0.1–0.3% and 1.7% [5,6]. Women are more affected than men, with a female/male ratio of 6:1. Genital female LS has generally two peak ages of presentation, in the prepuberal and postmenopausal years, but many cases are frequently described during reproductive life [3,7].

LS is a chronically relapsing disease, with a potential for atrophy, presenting multiple scarring leading to the destruction of the anogenital structures, such as fusion of labia minora, narrowing of the vaginal introitus and burying of the clitoris. The diagnosis is usually clinical, with important symptoms such as itching, pain, soreness, burning, dyspareunia and dysuria. The skin and mucosae appear covered by a

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white epithelium with mild or severe hyperkeratinization, atrophic papules and secondary features such as hemorrhagic, purpuric, hyperkeratotic, oedema, eroded or ulcerated lesions [8–11]. Biopsy of the initial lesion for definitive diagnosis and long-term follow up of affected patients are well established in the management of lichen sclerosus [12], but histological results need to be interpreted in association with clinical findings. Histological criteria include epidermal atrophy with hyperkeratosis, dermo-epidermal detachment, diffuse fibrosis with oedema and a marked characteristic “band-like” inflammatory infiltrate along the dermal-epidermal junction. The presence of high caliber vessels in the superficial dermis is frequently observed [13].

Anogenital LS is supposed to evolve to invasive vulvar carcinoma in 4–6% of the cases, implying the need of life-long follow-up in these patients. However as far as 60% of squamous cell vulvar carcinomas occur on a background of LS. Furthermore the association between LS with “differentiated type” vulvar intraepithelial neoplasia (VIN) has recently been described [1,14–18].

All anatomical damages lead to multiple functional impairments mostly interfering with sexual function and self image. For these reasons LS has a huge impact on a person's quality of life with important psychological and emotional consequences.

LS first-line treatment is through topical potent and ultra-potent corticosteroids [19–21]. Clobetasol propionate, the most commonly used steroid, may lead to an improvement of symptoms in almost all patients, complete relief of symptoms in about 60–70% and complete remission of skin changes in only 20% [22]. Other therapeutic options for LS include topical testosterone, now abandoned because of ineffectiveness and adverse effects (acne, severe hirsutism, clitoridomegaly) [23]. Surgical approach is limited to severe vaginal introitus stenosis, urinary retention, synechiae and squamous cell carcinoma. All these procedures, which may be useful to solve anatomical problems, are limited by the fact that they may damage tissues, create scars and present high recurrence rates.

Even when standard therapy with ultrapotent corticosteroids seems to solve main symptoms, anatomical damages such as atrophy, scarring and associated pallor usually tend to persist over time. For all these reasons we focused on a different therapeutic approach in order to face the problem of irreversible tissue damages.

The first and unique study regarding a new regenerative approach in LS to relieve symptoms, reduce atrophy and improve the mucocutaneous trophism has been published by the Italian group of Casabona in 2010. The treatment proposed was based on fat grafting and platelet-rich-plasma with satisfying results [24].

Fat grafting is a widely spread technique, currently adopted in reconstructive surgery with the aim of restoring volume, function and tissue disorders [25–30,36,37,40].

The first reports of fat grafting date back to the plastic surgery approaches of Neuber and Czerny [30], but the procedure was first standardized by Coleman [31–33]. Since then, several studies shed light on the clinical application potential and the biological properties of the fat tissue [25]. Notably, recent reports showed that adipose tissue has both immunomodulatory and anti-inflammatory properties. Dayer and coll. found that white adipose tissue is the main source of interleukin IL-1Ra, a natural antagonist specific to the proinflammatory cytokine IL-1 [34]. Such potentialities mainly depend on the activity of mesenchymal stem cells (MSC) residing in the stromal vascular fraction of injected fat tissue, which may modulate immune cell responses from a pro-inflammatory to an anti-inflammatory environment by interacting with dendritic cells, T lymphocytes and natural killer cells [35]. MSC may also exert immunosuppressive functions by secreting immunomodulatory factors (Transforming Growth Factor, Hepatocyte Growth Factor, Vascular Growth Factor, Endothelial Growth Factor, Fibroblast Growth Factor, Keratinocyte Growth Factor, Platelet Derived Growth Factor IL-6, IL-10 and HLA-G5, among others) which turn off the T-cell surveillance and the chronic

inflammatory processes [36]. The ultimate effect is an inhibition of fibrosis and the promotion of healing with beneficial effects in remodeling the extracellular matrix.

The rationale of this regenerative approach is the presence of stem cells progenitors within adipose tissue capable to differentiate into different mesenchymal tissues and to produce anti-inflammatory and immunomodulatory effect. [34].

The aim of this experimental study was to evaluate the efficacy of fat grafting in the treatment of vulvar LS.

Our primary outcome was to assess the efficacy in the improvement of vulvar mucocutaneous trophism and elasticity, the resolution/reduction of symptoms, while comparing the histological modifications of the vulvar skin before and after treatment. The secondary outcome was to evaluate the improvement in life quality, resumption of sexual activity and quality of sexual life.

2. Materials and methods

Upon Ethics Committee approval (LIVU 11.2011) and informed consent by the patients, we enrolled 46 women with severe LS of the vulva at the Preventive Gynecologic Oncology Unit of our Institution, between November 2011 and February 2014.

Inclusion criteria were: age between 25 and 80 years, a histopathologic diagnosis of lichen sclerosus, good general health conditions, failure of previous first-line treatments. Information was collected on: age, main symptoms and their duration, menopausal status, clinical aspects of the lesions, main location, impact on life quality and impact on sexual function. Impact on quality of life was assessed by Dermatology Life Quality Index (DLQI) and the modification in sexual function by Female Sexual Function Index (FSFI), dispensed at initial visit and six months following treatment. The need of steroids before and after fat grafting was accurately investigated in each patient.

A detailed clinical and vulvoscopic examination was performed before and after the procedure, taking into account: trophism of the skin and mucosae, caliber and elasticity of the vaginal introitus, clitoris burying degree, volume of labia majora and minora, scratching lesions, and white lesions. Each of these parameters was evaluated as mild, moderate, and severe.

We performed from one to three procedures in each patient, depending on the severity of the disease, complete or partial resolution of clinical findings, and patient's satisfaction and relief of symptoms. All procedures were performed using Coleman's technique in day-surgery regimen under local anesthesia, by one single plastic surgeon and only single gynecologist. Injections with mepivacaine/epinephrine were practised in the vulvar area and at the donor site (abdomen or thigh). Procurement was done by a 2 mm cannula with 4 holes on one side, on a 10 cm³ syringe, obtaining a low pressure (never exceeding 3 cm³) in order to minimize cell damage. Syringes were left decanting for approximately 3 min and then, after removing liquid of infiltration were put in the centrifuge, at a speed of 2800 rpm for two minutes. Following removal of infranatant fluids and supernatant oils, purified fat tissue was transferred into a 2.5 cm³ syringe. With an 18 g needle on a 2.5 cm³ syringe we performed a closed debridement, progressive and multilayer, without producing interspaces bigger than 2 cm in order to avoid injury of vascularization, and to provide a greater contact surface with vascular bed. After debridement we proceeded to progressive injection of adipose tissue. We started from the surface until fascial plane. The amount of adipose tissue could vary depending on clinical conditions (from 8 to 15 cm³ each side) particularly based on dimension of the vulva and tissue compliance with great care in avoiding tissue tension. Antibiotic prophylaxis preceded surgical procedures and all patients were instructed to follow appropriate use of emollients in the form of creams or gels during the next ten days after treatment. Massages with inert preparations were advised to stimulate hydration and ameliorate elasticity. A rest week was recommended after treatment, in order to let the adipose tissue settle without mechanical

disturbancies. For the same reason patients were advised to suspend sexual intercours for about twenty days.

Women were recalled one month, three months and six/twelve months after treatment, and a clinical and vulvoscopic assessments were conducted. A punch biopsy was performed after the last procedure in all cases (8 months later on average). The mean follow up period was 24 months.

Ten women were excluded from the study because of their severe clinical picture requiring vulvar flap reconstruction to restore vulvar anatomy with a different surgical approach.

Pre-treatment and post-treatment histological specimens were evaluated in our Pathology Department. We collected formalin-fixed, paraffin-embedded tissue samples from pre-treatment and post-treatment biopsies, and hematoxylin-eosin stained slides from all cases were evaluated by two pathologists (FMC and ADG).

We chose to evaluate 7 histological parameters corresponding to peculiar histological features of vulvar lichen: epidermal atrophy, hyperkeratosis, dermo-epidermal detachment, dermal inflammation, dermal homogenization (fibrosis), presence of oedema and presence of large caliber vessels in the superficial dermis. Each one of these parameters was assigned a score ranging from 0 (absent), 1+ (<25%), 2+ (between 25 and 50%), and 3+ (> 50%).

To better assess some of these parameters we performed histochemical and immunohistochemical stainings, particularly anti-CD34 to determine the vascular component, immunohistochemical staining anti-LCA to highlight inflammatory infiltrate and Masson histochemical staining to evaluate dermal fibrosis. For immunohistochemistry, five micron section were cut and placed on SuperFrost Plus (Thermo Scientific, Menzel Glaser, Germany). CD34 antibody (clone QBEnd 10) and LCA antibody (clone 2B11 + PD7/26) successfully used in diagnostic routine, were provided by DAKO. Immunohistochemistry was performed using the automatic system BenchMark XT (Ventana Medical Systems, Inc. Tucson, Arizona, U.S.A.). Reactions were revealed using the UltraView™ Universal DAB, a biotin-free, multimer-based detection system, according to the manufacture's instruction.

Data were analyzed using the SPSS 11 statistical package (SPSS, Chicago, IL). Comparisons were performed with the paired Student t-test. Statistical significance was set at 0.05.

3. Results

36 out of 46 patients were included in the study, with a mean age of 54 years (range, 25–80 years). 18 out of 36 (50%) were in menopause.

21 out of 36 women underwent one fat grafting (58%), 14 two fat grafting (39%) and 1 needed a third procedure (2%).

No complications were observed during and after the procedure. 70% of patients had no pain in the postoperative course, while in 30% pain gradually disappeared during the following ten days.

3.1. Macroscopical findings

From a clinical and vulvoscopic perspective 34 out of 36 patients showed a better vulvar trophism of the skin and mucosae (94%); 27 had an improvement in caliber and elasticity of the vaginal introitus (75%); clitoris burying degree was reduced in 18 cases (50%); 30 women reported an increased volume of labia majora and minora (83%); in 34 women a complete disappearance of scratching lesions was observed (94%); and 28 patients showed remission of white lesions (78%). Eventually, 34 patients stopped the routine use of topical corticosteroids (95%). **Table 1** shows clinical patterns before and after the procedure.

3.2. Subjective findings

Improvement in life quality was significant both for DLQI ($p < 0001$) and FSFI ($p < 0001$) after the analysis of scores given in the questionnaires.

Table 1
Clinical patterns before and after fat grafting.

Clinical pattern	Mild		Moderate		Severe	
	Before	After	Before	After	Before	After
Atrophy of skin and mucosae	0	18	10	10	26	2
Reduction of the vaginal introitus: caliber and elasticity	2	17	13	15	21	4
Clitoris burying degree	1	4	3	15	32	17
Volume reduction of labia majora and minora	2	12	10	20	24	4
Scratching lesions	0	30	30	6	6	0
White lesions	13	31	13	5	10	0

3.3. Microscopical findings

A marked reduction in hyperkeratosis was histologically detected in 67% of patients. Dermo-epidermal detachment was observed in only 1 pre-treatment biopsy sample, and it disappeared in post-treatment evaluation. A reduction of chronic inflammation was documented in 89% of the cases, while a reduction of fibrosis in 67%. A disappearance of large caliber blood vessels with formation of smaller ones was seen in 44% of cases, probably as a reaction to the lipofilling procedure. A slight reduction of dermal oedema was observed in 33% of patients. **Figs. 1 and 2** illustrate histological and clinical results respectively, in a same patient before (A) and after (B) treatment with fat graft.

4. Discussion

Prevalence and incidence rates of LS vary considerably, but the disease can be very impactful especially in women during reproductive age, since it may interfere with active sexual life and fertility desire.

First line therapy with high-potent topical corticosteroids reduces symptoms in the majority of patients. Treatment is safe and effective, without evidence of notable steroid-related damage following appropriate use [23]. However the continued use of corticosteroids presents some limitations. First of all about 30–40% of patients still present symptoms after therapy [20] and need to continue steroid applications for long periods of time. Furthermore, LS is a skin condition characterized by skin atrophy, scarring and hypopigmentation, which may persist after treatment with steroids, so that in many cases clinical resolution is not satisfactory. These women will continue to have problems related to LS such as inadequate sexual function or anatomical impairment. In some instances steroids can even aggravate clinical picture by worsening atrophy.

Bearing this in mind, we focused on a different therapeutic method using fat grafting, in order to provide an alternative approach when first line treatment is not efficacious, as well as to support standard treatment whenever a complete resolution of symptoms cannot be obtained, and to reduce atrophy caused by both disease itself and steroids.

Fat grafting is currently a very important tool in reconstructive medicine thanks to its regenerative properties.

Many pathologies already benefit from fat grafting: burns sequelae, radiotherapy tissue damage, reconstruction after oncological surgery, improving facial nerve function [28–30].

LS is as a clinical condition that needs tissue regeneration, and thus can benefit from fat grafting. Another regenerative technique described in literature is Platelet Rich Plasma injection (PRP), currently used in several reconstructive medical fields, such as maxillofacial and orthopedic surgery, with good results. PRP approach finds its rationale in the presence of different growth factors released by platelets which promote tissue repair and influence in angiogenesis and inflammation reduction [38,39].

Casabona et al. [24] first proposed the use of both PRP and fat grafting in the treatment of LS with favorable outcomes. In this study we used fat grafting alone, with good results. The use of fat grafting

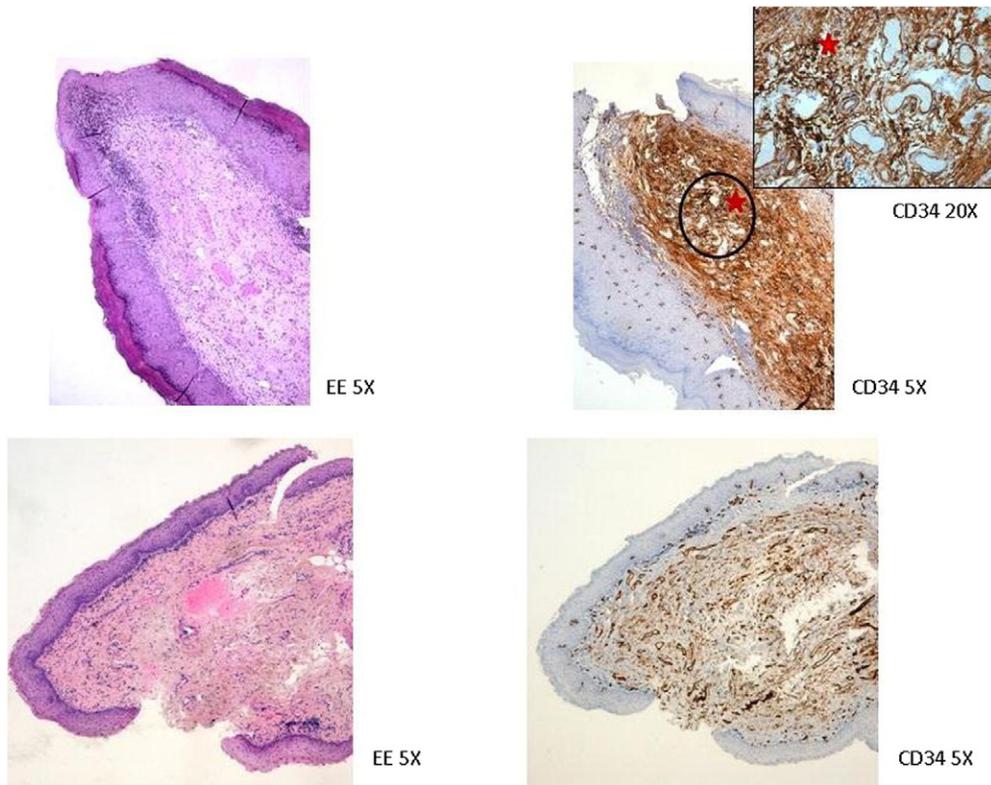


Fig. 1. Histological pictures in a pre-treatment (A) and post-treatment (B) biopsy sample, after staining with hematoxylin-eosin (on the left) and anti-CD34 antibody (on the right). Together with a marked reduction in hyperkeratosis, fibrosis and inflammation, immunohistochemical staining shows how large caliber vessels (brown) disappear in the post-treatment biopsy sample (original magnification for both hematoxylin-eosin and CD34 stainings: 5 \times).

together with PRP makes the procedure very long and complex. Indeed PRP processing technique requires a high level laboratory centrifuge, because of the labor intensive process, generally requiring two spins and multiple transfers.

In our study, 94% of patients showed a better vulvar trophism, 75% had an improvements in caliber and elasticity of vaginal introitus and 83% reported an increased volume of labia majora and minora. These

anatomical improvements represent per se a good chance for women to replace their “original” vulvar anatomical appearance.

Furthermore in our population, 50% of cases were premenopausal women: these patients need treatment to be directed not only towards symptoms, but also to be able to restore their sexual activity, which is often compromised and rarely taken into consideration. We reported statistically significant outcome ($p < 0.05$) in recovery and improvement

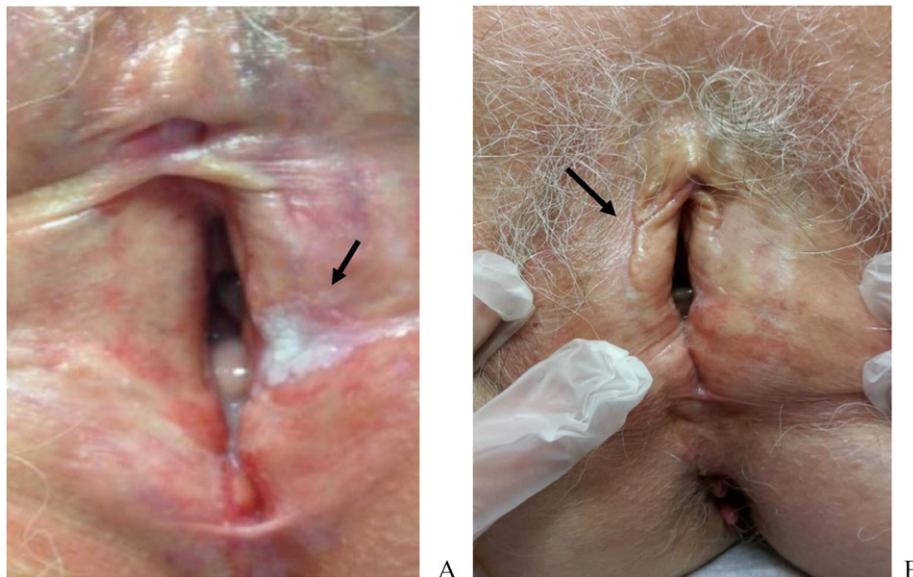


Fig. 2. Clinical pictures showing disappearance of white lesions, volume increase of labia majora and minora, reduction of atrophy, increase of elasticity of skin and mucosae, pre-treatment (A) and post-treatment (B).

of sexual activity, and consequently in quality of life. Our results underscore the possibility of simplifying such a complex technique. We are confident that simplifying and standardizing fat graft technique may offer an important mini-invasive approach to selected cases of LS, to be currently used in clinical practice.

This is the first experimental study about fat grafting alone in the treatment of LS. The strength of our study is the absence of intra- and post-operative adverse events and the encouraging results concerning improvement of symptoms, impact on life quality, clinical picture and histology. The weakness of the study is the small sample size, since we included only the most severe cases of LS.

This is the first study in which pre- and post-treatment biopsies were evaluated according to precise histological criteria, both with classical hematoxylin-eosin staining and using specific antibodies to better highlight some peculiar characteristics of vulvar LS. We observed remarkable histological improvement in terms of skin and dermal trophism in all cases, using specific parameters.

Our experience can be considered a pilot-study, to be confirmed and validated in a wider sample of women. A longer follow up is also needed, in order to assess the necessity of boosters. A limit of this procedure is that a day surgery operating theater with its related costs, is required. Another limitation is the need of both a gynecologist and a plastic surgeon, requiring a highly specialized structure, making the procedure difficult to be extended.

In conclusion, despite our encouraging results regarding an innovative approach to LS, corticosteroids remain the first line therapy until more data will be available. We propose fat grafting as a support and completion therapy only in selected women affected by LS, who are poor or non responders to first line therapy or in cases in which the anatomical impairment does not allow a regular sexual function and a good quality of life.

Conflict of interest statement

The authors have none to declare.

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