



Study of ultrastructural changes of the non-neoplastic epithelial disorders of vulva after focused ultrasound treatment

Cheng-zhi Li[†],
Xiaogang Liao,
Kejian Wang,
Yunbo Tian,
Niangang Yin &
Zhi-biao Wang

[†]Author for correspondence
Biomedical Engineering
Department, Institute of
Ultrasound Engineering in
Medicine, PO Box 153,
1 Yi Xue Yuan Road,
Yuzhong District, Chongqing
400016, China
Tel.: +86 236 788 6195;
Fax: +86 236 861 0718;
E-mail: foreigners@
haifu.com.cn

Objective: This study was undertaken to investigate the ultrastructural changes of non-neoplastic epithelial disorders of the vulva (known as vulvar dystrophies) after focused ultrasound treatment and to assess the effectiveness of such treatment. **Materials & methods:** In total, 60 patients with non-neoplastic epithelial disorders of the vulva were enrolled in this study and treated by focused ultrasound therapy. Before and after treatment, changes to ultrastructures in the treatment region were evaluated using pathological and electron microscopic studies in 30 patients and the short-term treatment effectiveness was assessed. **Results:** Symptoms of the patients were greatly reduced or even eliminated. There was no itching or pain during sex. Lesions prior to treatment showed various degrees of hyperplastic atrophy and degenerative changes. At the same time, an increase in epithelial keratotic and decrease in melanotic granules and desmosomes was observed. Mitochondria with swelling and capillary contraction were noted. After the focused ultrasound treatment, the epidermis and dermis showed signs of recovery at various levels. The stratified squamous epithelium of vulva had recovered normal stratification and thickness. The pigmentation cells could be obviously seen in the basal layer. **Conclusions:** The ultrastructural changes of non-neoplastic epithelial disorders of the vulva indicated the disappearance of symptoms of the disease and recovery of the ultrastructures. These results indicate that patients with vulvar dystrophies can be treated with focused ultrasound effectively.

Non-neoplastic epithelial disorders of the vulva [1,2], also called vulvar dystrophies, are a chronic skin disease characterized by signs and symptoms, such as skin depigmentation and rhagas, atrophy of the vulva and a narrow vaginal orifice from which vulval pruritus and pain during sex may arise. It is a refractory disease in gynecology and difficult to cure, since the definite etiology still remains unclear. Some remedies, such as hormone and herbal medications and laser or microwave exposure, have been used to treat this disease, but high recurrence rates are found [3–6].

Focused ultrasound treatment appears to be a new therapeutic modality, in which the physical properties of a focused ultrasound beam allow it to be targeted accurately at the dermis layer to destroy the disease by using thermal and cavitation effects. This method has been used to treat vulvar dystrophies in 76 Chinese patients successfully [8]. Signs and symptoms of patients were eliminated and the recurrence rate was satisfactory. Nevertheless, further pathological studies are still necessary to confirm the treatment effects after focused ultrasound treatment. In our research, we studied the biopsies of the

vulvar skin of 60 patients before and 3–6 months after focused ultrasound treatment, using pathological and electron microscopic observations and evaluations.

Materials & methods

Patients

A total of 60 patients with vulvar dystrophies, aged 17–70 years with a mean age of 43.45 years, were enrolled in this study and underwent focused ultrasound treatment from June 2000 to December 2002. The courses of disease of all patients were 5 years for half the patients and 10 years for the other half. It was pathologically diagnosed that, among the 60 patients, 31 had lichen sclerosis and the remaining 29 had squamous cell hyperplasia. Both types were accompanied by slight or mild atypical hyperplasia. Before treatment, informed consent was obtained and the ethical committee of the university approved the study.

As previously described [8], inclusion criteria for patients included pathologically diagnosed vulvar dystrophy, no other therapies used within 3 months of ultrasound therapy, and at least two of the following signs or symptoms:

Keywords: focused ultrasound treatment, nonneoplastic epithelial disorders of vulva, ultrastructural changes, vulvar dystrophy

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- Vulvar pruritus
- Burning or stimulating sensation
- Skin coarsening
- Rhagades or disruption
- Skin depigmentation and leukoplakia
- Atrophy of the nymphae or clitoris

Most patients in this study had previously received local treatment, such as hormone and herbal medications and laser or microwave exposures, 3 months before ultrasound therapy. Patients who were pregnant or lactating or who had acute vaginal infection were excluded from this study.

Treatment procedure

All patients were treated with a Model CZF-focused ultrasound therapeutic device, developed and manufactured by Chongqing Haifu (HIFU) Technology Co. Ltd. The device is composed of a user console, a main system, an electric control part, a water cabinet and a treatment applicator, as described previously by us [8]. The treatment applicator was designed using the focusing lens theory with a focal volume of $1.1 \times 1.1 \times 4$ mm, with frequencies ranging from 5 to 8 MHz and a maximal output acoustical power of 5 W. The

main system provides output electricity to the power source that transforms the electricity into ultrasonic beams. An ultrasonic beam is focused at the target deep in the tissue to heat the lesions.

Routine disinfectant procedures were given before treatment. Patients received general anesthesia by an intravenous injection of disoprolol (Abbott Laboratories, IL, USA) and fentanyl citrate (Yichang Homanwell, Yichang, China) or local anesthesia with Anestacon® (ledocaine, lignostab, lidocaine; Xinan Pharmaceuticals Co. Ltd. Chongqing, China) during the procedure. The treatment applicator directly contacts the skin coupled by purified water from the applicator. A continuous scanning mode was adopted at a scanning speed of 2–3 mm/s for a period of 15–60 min during each treatment session, according to the sizes of lesions, as depicted by Li and colleagues [8]. Post-treatment management, such as ice application, was used to relieve the irritation if necessary.

Effect evaluation

Before and after treatment, patient symptoms, including pruritus and sexual satisfaction, were investigated by a questionnaire. Scores on the questionnaire and recovery of body signs and symptoms were used to evaluate effectiveness.

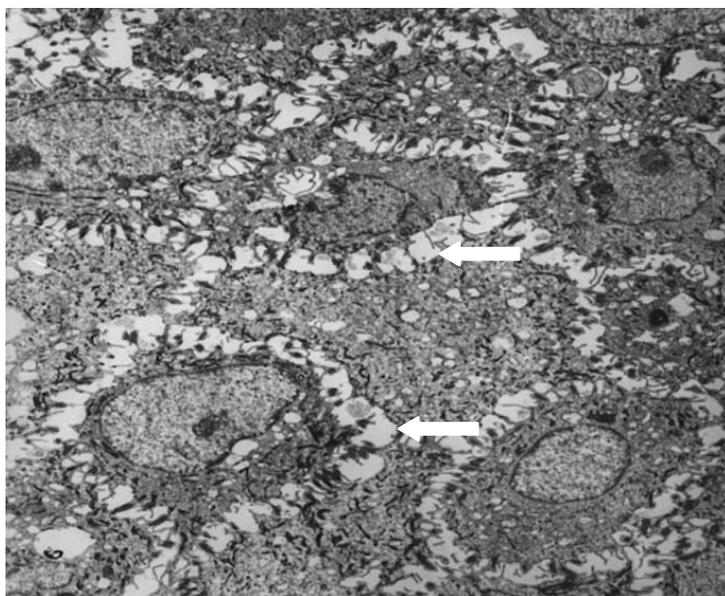
Follow-up

Multisite biopsies of all patients from the rhagades, ulceration, swell, knot or coarse points of the vulva were obtained for light and electron microscopic examinations before treatment. All patients were followed after focused ultrasound treatment. Half of the 60 patients were followed 3–6 months after the therapy, and their biopsies of the treated region were taken for light and electron microscopic examinations.

Preparation of electron microscopic samples

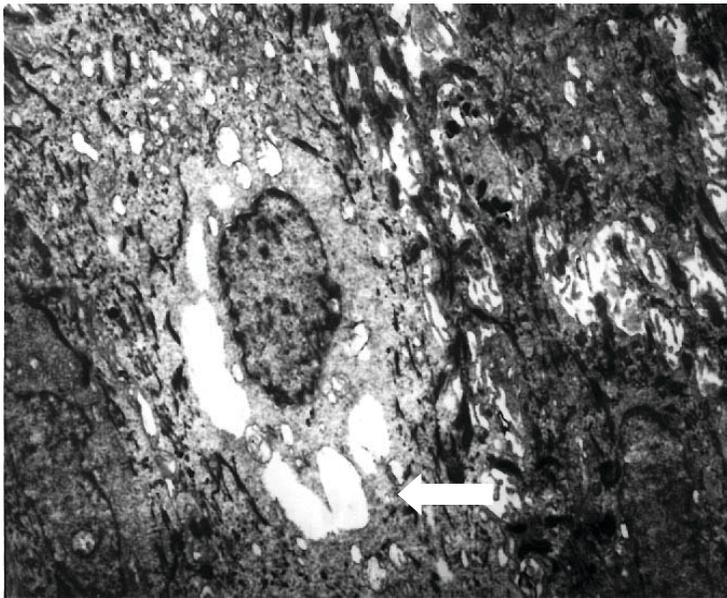
Tissue samples 1 mm³ in volume were prefixed in 4% glutaral solution (pH = 7.4) for 2 h and then fixed using 1% osmium tetroxide solution and gradually dehydrated with ethanol and propanone. After embedding by epoxy resin 618, tissue samples were sliced into 1- μ m thick slices, stained by toluidine blue and observed under light microscope. An Ultracut E ultramicrotome was used to prepare ultrathin sections, which were stained by acetic acid and lead citrate and observed under a Hitachi-600 electron microscope.

Figure 1. Before treatment, the intercellular space between stratum spinosum cells increased, the desmosome count reduced and the nuclei forms were normal.



Multiple cavitations (arrows) formed around the nuclei or the local cytoplasm dissolved and mitochondrion had swelling or cavitation at various levels. Microfilament count was not sufficient. Magnification $\times 3500$

Figure 2. Before treatment, vacuolation formed surrounding the nucleus and the local cytoplasm dissolved.



Mitochondrion swelling or vacuolation (arrow) were found and there was few organelles in the cytoplasm, which lacked microfilaments. Magnification $\times 6000$.

Results

Short-term treatment effect of ultrasound therapy

Skin of the treated region turned slightly red and swollen without pain, but symptoms such as itching were eliminated just 0.5 h after treatment. Local swelling became more obvious 1–2 days after treatment, but there was still no pain or pruritus. After 3 days, local swelling was gradually reduced and disappeared completely after 7–10 days. Pruritus remained improved 1 month after treatment. Pain during sex was greatly reduced and even eliminated. According to our study of treatment effects between various pathological types, there was no significant difference in short-term results.

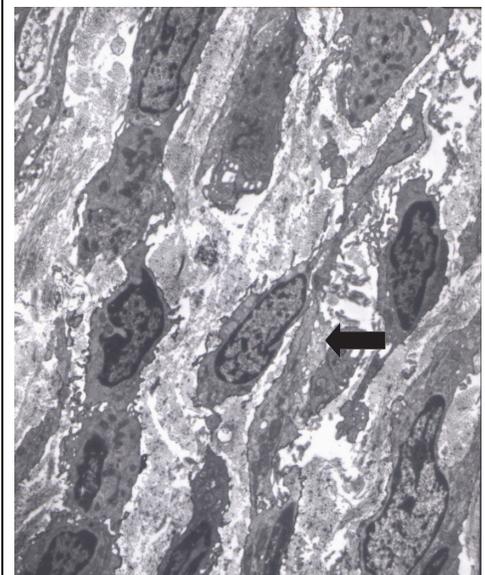
Light & electron microscopic examinations before treatment

Under light microscope, in 31 cases with lichen sclerosis, the epithelia of the diseased skin became thin and had excessive cornification. The basal cells were liquefied and degenerated, melanocytes were reduced and the epithelial ridge became blunt or disappeared. The superficial layer of dermis had edema, and homogenization occurred owing to disappearance of collagenous fibrosis structures, leading to reduction or absence of elastic fibers and infiltration

of inflammatory cells at various layers in the dermis. In 29 cases with squamous cell hyperplasia, epithelial cells of the diseased skin had excessive cornification, the prickly cell layers were thickened and the epithelial ridge was extended. Melanin deficiency in the basal cell layer was frequently seen. In the dermis layer, there was nonspecific chronic inflammation and the count of microvessels was reduced.

Furthermore, under electron microscope, in 31 cases with lichen sclerosis, epithelial atrophy of the diseased skin was observed with excessive cornification. The intercellular space of the stratum spinosum was enlarged. It was also observed that the desmosome count was reduced, but nucleus formation was normal. However, vacuolation was formed surrounding the nucleus and local cytoplasm was dissolved; mitochondrion swelling or vacuolation was observed and there were few organelles in the cytoplasm, which lacked microfilaments (Figure 1). Melanin granules in basal layer cells were notably reduced, or even disappeared, and melanocytes were rarely seen (Figure 2). Collagenous fibrils in the dermis did not align in order, few fibroblasts were observed and the microvessel count was reduced. Microvessels were constricted or contracted (Figure 3) and nerve endings were scarcely seen. At

Figure 3. Before ultrasound treatment, collagenous fibril in the dermis aligned in disorder.



Besides the disordered alignment of collagenous fibrils (arrow), the fibroblast count was few and the microvessel count was reduced. Magnification $\times 3000$.

the same time, a mass of mature lymphocytes infiltrated the epidermis and dermis. In 29 cases with squamous cell hyperplasia, the epithelial cell count, particularly prickle cell layer cell count, increased obviously, the cuticular layer was thickened and the desmosome count decreased. The ultrastructure of the epidermis also had pathologic changes at different levels. Collagenous fibrils in the dermis were aligning in disorder and slight lysis was observed. The fibroblast count was reduced and the dissepiment of microvessels was thickened. Mature lymphocyte infiltration was also seen in the epidermis and dermis. In all 60 cases in our study, the severity of the ultrastructure pathological changes was closely relevant to the disease course and conditions.

Light & electron microscopic examinations after treatment

In light microscopic examination of all cases after treatment, it was found that the stratified squamous epithelium of the vulva recovered. No excessive cornification or thickening or thinning of the prickle cell layer was found. The epithelial ridge recovered and depigmentation of basal layer cells was observed. Inflammatory infiltration in the dermis was notably reduced obviously, and even disappeared. The microvessel count in the dermis increased.

During electron microscopic examination of all cases after focused ultrasound treatment, it was found that the diseased skin demonstrated recovery at different levels. Epithelial cells were aligning in order with distinct stratification (Figure 4). Rich free ribosomes were found in basal layer cells and the mitochondrion count increased. Melanin granule count and hemidesmosome count also increased. Desmosome structures in the echinocytes were well developed with normal nuclei. Rich microfilaments, mitochondrion and endoplasmic reticulum were observed in the cytoplasm. The melanin granule count also increased at different degrees (Figure 5). The fibroblast in the dermis and microvessels increased and cavity of vessels basically recovered (Figure 6). Nerve endings in the deep region of the dermis increased, while infiltrative lymphocytes in epithelia and dermis were markedly reduced, and even disappeared.

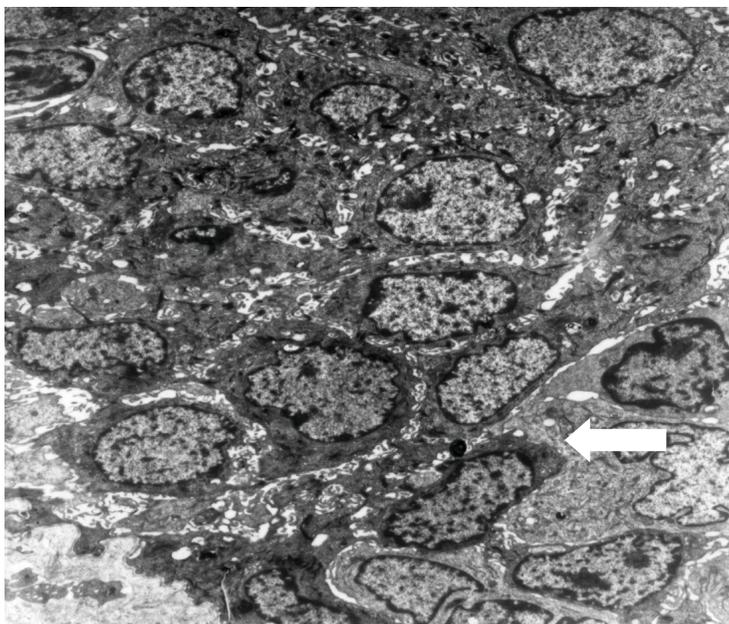
Discussion

Pathogenesis of non-neoplastic epithelial disorders of the vulva

Non-neoplastic epithelial disorders of the vulva or vulvar dystrophies are a secondary degeneration of the epithelia caused by pathologic changes of the microvessels in the dermis [7,9]. The results from the 60 cases in our study demonstrated that pathologic changes of the disease were characterized by the spasm and reduction of tiny capillaries in the dermis, swelling and vacuolation of mitochondrion in epithelia, broadening of the intercellular space and reduction of desmosome structures. A desmosome is a cell-connective structure of epithelial cells. Its reduction leads to brisement of keratinocytes and the formation of epidermal inner crevices and blister [10,11].

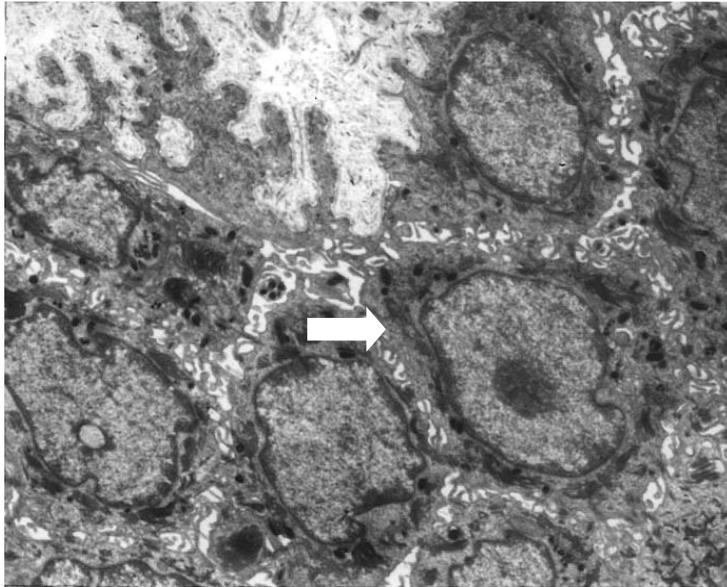
Comparison and observation of ultrastructures before and after focused ultrasound treatment further supported the viewpoint that non-neoplastic epithelial disorders of vulva would cause degeneration of the cell's energy supply due to microcirculative disorders. Disorders of microcirculation may lead to anoxia of histocytes, influence the function of tyrosinase, reduce synthesis of melanin and finally cause depigmentation and blanching of the skin. Additionally, microcirculative disorders might lead to secondary inflammation and release inflammatory media and stimulate nerve endings to arouse vulval pruritus.

Figure 4. After ultrasound treatment, diseased skin showed recovery at different levels.



Epithelial cells aligned in order with distinct stratification (arrow).
Magnification $\times 3000$

Figure 5. After ultrasound treatment, desmosome structures in echinocytes were well developed with normal nuclei.



Rich microfilaments, mitochondrion and endoplasmic reticulum were seen in the cytoplasm (arrow). The melanin granule count also increased at different degrees. Magnification $\times 6000$.

Mechanism of focused ultrasound treatment

As a recently developed noninvasive technique, focused ultrasound therapy has been used to treat benign and malignant tumors in human patients [12–16]. On the basis of numerous animal trials [17], focused ultrasound therapy has been used to treat dystrophies of the vulva in Chongqing since 1999, and encouraging preliminary results have been obtained [8].

In ultrasound therapy, an ultrasound beam is directed to the dermal layer of the skin. This may give rise to thermal effects and cavitation, which causes damage to the microvessels and subsequently improves growth and reconstruction of the microcirculation of the treatment area. Owing to the destruction of the endothelial cells, the permeability of capillaries was evidently increased. As a result, transient congestion and edema were observed after therapy, but the epidermis in the treated region remained intact.

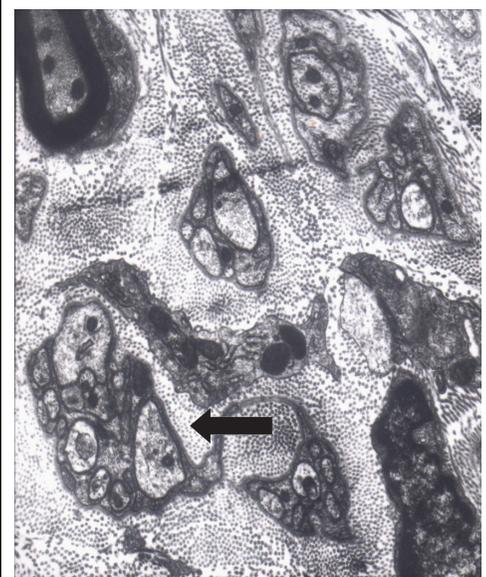
It was found that ultrasonic exposure might accelerate cell proliferation and increase the synthesis of collagen protein and angiogenesis factors interleukin-8, basic fibroblast growth factor and vascular endothelial growth factor, which suggested that ultrasound could stimulate cell proliferation, protein synthesis and revascularization. Recent studies suggested that the interaction

between an ultrasound beam and microbubbles in microvessels could enhance the permeability of cell membranes and lead to the collapse of microvessels less than $7\ \mu\text{m}$ in diameter and to broadening of the intra-epithelial cell space [18,19].

Therapeutic effect of focused ultrasound therapy

Focused ultrasound treatment uses acoustic properties focused at the dermis layer of the vulvar skin, producing a series of biological effects: improves the microcirculation in the dermis and nutritional supply to local tissue, and recovers the pigmentation and desmosome of the epithelia. Furthermore, after elimination of local inflammation, the stimulus of nerve endings was reduced and the pruritus symptoms decreased or disappeared. In our study, after focused ultrasound treatment, the microvessel count increased and blood vessel dissepiment and lumen recovered. Fibroblast and nerve ending count in the dermis was increased. The epithelial strata recovered to normal and aligned in order. The mitochondrion count increased and intracellular desmosome structure recovered to normal and the count increased. Melanin granules in cytoplasm increased at various degrees and melanocytes in the basal layer also increased. We also observed that the lymphocyte infiltration of local tissues reduced or disappeared.

Figure 6. After ultrasound treatment, the fibroblast cell count and microvesicle count increased.



The nerve endings in deep region of dermis are increased (arrow). Magnification $\times 3000$.

These ultrastructural changes led to the reduction or disappearance of signs and symptoms in patients. In all 61 patients, just 30 min after therapy, elimination of itching was reported. In some patients, white lesions with pigmentation found in the vulva were decreased gradually and disappeared 6 months after treatment. Rhagas and atypical hyperplasia were also recovered and the skin elasticity gradually became normal 3–6 months after treatment. In the questionnaire, patients reported a decrease of pain or uncomfortable feelings during sex. These are believed to be relevant to ultrastructural changes after focused ultrasound treatment.

Conclusion

In conclusion, the ultrastructural changes of non-neoplastic epithelial disorders of the vulva indicated the disappearance of disease and recovery of the ultrastructures of the vulva. Focused ultrasound treatment is effective in treating non-neoplastic epithelial disorders of the vulva, pruritus of patients can be well controlled and diseased skin structures can also be rehabilitated. Although this study has validated from a cell level that focused ultrasound treatment is an effective method for vulvar dystrophies, further studies and long-term results are needed to explore its function on improving the biological effects of microcirculation and local tissue nutrition.

Executive summary

- Focused ultrasound has been used in clinical application to treat patients with vulvar dystrophies.
- The ultrastructural changes of vulvar dystrophies after focused ultrasound were assessed in this study.
- After focused ultrasound therapy, symptoms of patients were eliminated or reduced.
- The ultrastructural changes observed support the therapeutic results.
- The focused ultrasound therapy appears to be a promising option to treat vulvar dystrophies.

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