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Original Research

The efficacy of high-intensity, focused ultrasound treatment for non-neoplastic epithelial disorders of the vulva

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Abstract: Non-neoplastic epithelial disorders of the vulva (NNEDV) are common types of vulval lesions. Although corticosteroids represent a first-line treatment for NNEDV, concerns exist about the safety associated with long-term topical corticosteroid use. Recently, several clinical trials have identified high-intensity focused ultrasound (HIFU) as a promising treatment modality for NNEDV. The aim of this multi-center, randomized, controlled clinical trial was to investigate the efficacy of HIFU therapy in women with NNEDV based on histological alterations. We enrolled patients who were clinically diagnosed with NNEDV. They were randomized into 2 treatment groups: 1) halcinonide for 3 months or 2) HIFU once. A total of 123 patients were biopsied both prior to and after the therapy, and 62 and 61 patients were assigned to the HIFU and halcinonide groups, respectively. The histological changes were then analyzed. After the treatments, the therapeutic effects were observed in both groups. Comparing the diagnosis and alterations in lichenoid and sclerotic patterns and in chronic inflammation, we found statistically significant differences. Furthermore, when compared with the halcinonide group, the HIFU group exhibited enhanced curative effects that were statistically significant (P = 0.039). Based on the histological evidence from this randomized, controlled trial, HIFU represents an effective method for the treatment of NNEDV.

Key words: Non-neoplastic epithelial disorders of vulva, high-intensity focused ultrasound, pathology.

Introduction

Non-neoplastic epithelial disorders of the vulva (NNEDV) comprise a heterogeneous group of vulval lesions, including lichen sclerosus, lichen planus, and lichen simplex chronicus (1). The lesions are common and can induce pruritus and cause pain. Lichen sclerosus (LS) is associated with an increased incidence of Squamous cell carcinoma (2). Because the etiologies of these disorders remain unclear, effective treatments are currently lacking. Topical treatment of the lesions with corticosteroids, which has been widely accepted, provides prompt symptomatic relief and inhibits cellular proliferation (3, 4). However, safety concerns exist regarding the long-term usage of topical corticosteroids. Moreover, the recurrence rate after corticosteroid treatment is high (2, 5).

Although ultrasound currently represents an essential diagnostic method, its therapeutic applications are not well recognized. High-intensity focused ultrasound (HIFU) therapy can transport energy in the form of ultrasound waves through a layer of intervening tissues to specific the target points within body organs. In the process, the transported energy increases the temperature and brings other biological interactions of the target tissues without damaging surrounding or overlying tissues (6). Because of its non-invasive nature, this technology has been used successfully for the ablation of solid tumors (7-9). Furthermore, HIFU has been used for hemostasis, thrombolysis, targeted drug delivery, and several other therapeutic applications (6). Recently, several clinical trials have highlighted HIFU as a promising treatment modality for NNEDV (1, 10). HIFU treatment relieved vulval pruritus and recovered the elasticity and appearance of the vulval skin (10). However, these reports were evaluated based on symptoms and skin appearance. Until now, there has been no evaluation of the therapeutic response based on histology, which reflects the pathogenesis of the disease.

The aim of this study was to investigate the efficacy of HIFU therapy in women with NNEDV. The evaluation of therapeutic response was based on histological alterations prior to and after HIFU therapy compared to corticosteroid therapy.

Materials and Methods

Patients and clinical data

This study was a multi-center, randomized, controlled clinical trial that included the Peking Union Medical Collage Hospital in Beijing, the Women's Hospital School of Zhejiang University in Zhejiang, the Southwest Hospital in Chongqing and the Peking University Shenzhen Hospital in Shenzhen. The study was registered with the Chinese clinical trial registry (number ChiCTR-TRC-09000434), and the study participants were randomized into either the HIFU or halcinonide (control) groups by the Chinese Cochrane Center. We enrolled patients who were clinically diagnosed with NNEDV from January 2009 to December 2010. All

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participants provided informed consent. The patients were treated with halcinonide for 3 months or once with HIFU. Among the enrolled patients, 123 were biopsied both prior to and after therapy. Sixty-two patients were assigned to the HIFU group while 61 patients were assigned to the halcinonide group. None of the patients had pre-existing cardiovascular or inflammatory diseases or diseases of the liver, kidney or reproductive system. The patients had no previous treatments within the 3 months prior to beginning the therapy. Biopsies prior to the treatment excluded patients with VIN.

Treatment methods

The HIFU machine used in this study was a Model CZF (Chongqing Haifu Technology, Chongqing, China). The transducer power was 3.5 watts, and the frequency was 9.05 MHz. The applicator was applied directly to the skin, using water as the coupling medium. After routine disinfection procedures were performed, the patients received local anesthesia with 2% lidocaine. At a speed of 4 mm/s, linear scans were performed over the lesion until the target area became mildly reddish and developed edema; some patients developed hard nodules. The treatment duration ranged from 5-10 minutes. The treatment areas included a 5-mm margin of healthy skin beyond each lesion. An oil gauze and ice bag were used to relieve the irritation during each treatment. Each patient got the treatment once. Twenty-four hours after each treatment, a 1:5,000 potassium permanganate solution sitz bath was administered twice daily. After 3 months of HIFU therapy, biopsies were obtained to assess the histological alterations as an evaluative index beyond signs and symptoms and the quality of life score.

Patients in the halcinonide group were treated with halcinonide, which was applied to the lesions twice daily. After the symptoms improved, the treatments were reduced to twice per week. The total treatment course lasted 3 months.

Histopathological evaluation of biopsy specimens

Biopsies were obtained prior to and after the treatments. All tissues were fixed in 10% formaldehyde and embedded in paraffin. The paraffin blocks were subsequently cut and immunohistochemically stained with hematoxylin-eosin. The pathological diagnoses were based on the classification designated by the International Society for the Study of Vulvovaginal Disease (ISSVD) in 2006 (11). The following 3 predominant altered histological patterns were analyzed: 1) the acanthotic pattern, which represents an increased number of epithelial cells; 2) the lichenoid pattern, which represents band-like lymphocytic infiltrate within the upper dermis, accompanied by epidermal basal layer damage; 3) dermal homogenization/sclerosis pattern, which represents the obliteration of boundaries between collagen bundles, showing a homogenized, "hyalinized" appearance of dermis. An additional 2 histological patterns that are commonly associated with ISSVD were also analyzed. Chronic inflammation was reflected by the appearance of chronic inflammatory cells infiltrating into the epithelium or dermis. Epithelial atrophy was reflected by the appearance of a thin epithelium with the loss of the epithelial ridge. Based on the altered histological patterns, 4 diagnoses were conducted. Lichen sclerosus (LS) was diagnosed with both lichenoid and sclerosis patterns. Lichen planus (LP) exhibited a lichenoid pattern without sclerosis. Squamous cell hyperplasia (SH) was diagnosed when there was an acanthotic pattern, with or without hyperkeratosis. Non-specific inflammation was reflected by the appearance of a dermis with non-specific chronic inflammatory cells infiltration, as well as a non-hyperplastic epidermis with or without hyperkeratosis.

Statistical analysis

Statistical analyses were performed using the SAS software, version 9.2 (SAS Institute, Cary, NC, United States). The patient ages in the 2 groups were compared using a *t*-test. We found that the disease duration data were not normally distributed; therefore, we used the Mann-Whitney U-test to compare the disease durations. Fisher's exact test was used to compare the diagnosis of the 2 groups prior to the treatments. A generalized linear mixed model was generated to compare the changes of each pattern and the diagnosis prior to and after the treatments. Diagnosis changes using different treatments were also compared using the same model, and $P < 0.05$ was considered statistically significant.

Results

The HIFU group included 62 patients ranging in age from 20 to 77 years (mean age 39.3 years). The disease duration ranged from 1 month to 35 years (median 3 years). The halcinonide group included 61 patients ranging in age from 22 to 88 years (mean age 44.4 years). The disease durations ranged from 5 months to 24 years (median 3 years). The patient ages in the 2 groups exhibited significant differences, but the mean ages were similar. The disease duration, as well as the diagnosis prior to the treatments, did not exhibit significant differences (Table 1).

Prior to the treatments, more than 30% of the cases

Table 1. Basic information of the patients in the 2 groups.

	HIFU group	Halcinonide group	P
Mean age ± SD, years	39.3 ± 12.1	44.4 ± 13.5	0.030
Median disease duration, years	3 (0.08~35)	3 (0.42~24)	0.098
Diagnosis			0.300
Non-specific inflammation	9 (14.5%)	16 (26.2%)	
SH	22 (35.5%)	23 (37.7%)	
LP	2 (3.2%)	2 (3.3%)	
LS	29 (46.8%)	20 (32.8%)	

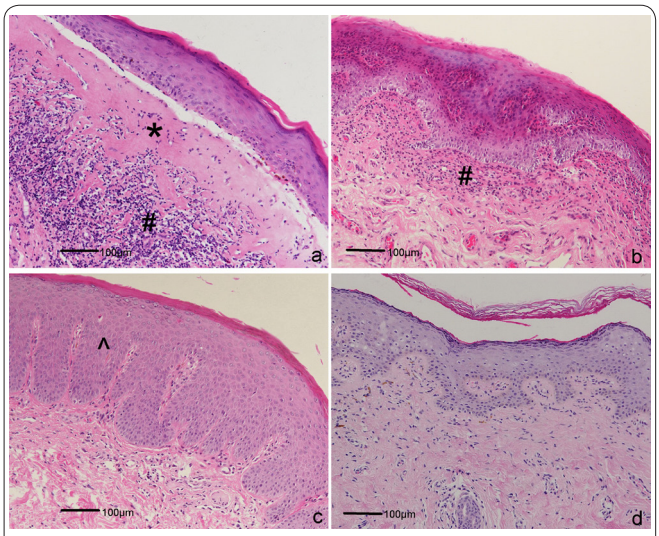

Figure 1. a. A case diagnosed as LS (case No. 11), exhibiting a hyalinized appearance of the upper dermis (*) with band-like lymphocytic infiltration within the dermis (#). The epithelium detached from the dermis because of the liquefaction and degeneration of the basal cell layer. **b.** A case of LP (case No. 28), exhibiting band-like lymphocytic infiltration within the dermis (#). **c.** Squamous cell hyperplasia (case No. 5), which exhibits a thickened epithelium (^) with an increased number of acanthocytes. **d.** Regression of LS after HIFU treatment, exhibiting normal dermis with scattered lymphocytes infiltrate (case No. 11, same patient with Figure 1a).

diagnosed as LS in both of the 2 groups exhibited homogenization and a hyalinized appearance of the upper dermis with band-like lymphocytic infiltration within the dermis. The epithelium often exhibited hyperkeratosis and sometimes became thin because of the loss of the epithelial ridge. The basal cell layer exhibited liquefaction and degeneration that promoted the detachment of the epithelium from the dermis (Figure 1a). LP revealed band-like lymphocytic infiltration within various layers of the dermis. We also observed liquefaction and degeneration of the basal cell layer, or lymphocytic infiltration into the epithelial and dermal interface (Figure 1b). Squamous cell hyperplasia resulted in epithelial thickening, with an increased number of acanthocytes (Figure 1c). The epithelial ridges were extended. Hyperkeratosis of the epithelium and variable extents of nonspecific lymphocytes infiltration were apparent.

After the treatments, therapeutic effects were commonly observed in both groups. The effects are shown in Tables 2 and 3. A comparison of the lichenoid, sclerotic patterns and chronic inflammation in both groups revealed statistically significant differences. After the HIFU treatment, the diagnoses of 9 LS patients changed to non-specific inflammation (Figure 1d), whereas 13 patients were diagnosed with SH. The diagnoses of 8 SH patients changed to non-specific inflammation. The diagnoses were statistically significant ($P < 0.0001$). Similarly, after halcinonide treatment, the diagnoses of 7 LS patients changed to non-specific inflammation, whereas 6 changed to SH. Four additional SH patients had their diagnoses changed to non-specific inflammation. In the halcinonide group, the diagnoses also showed significant differences ($P = 0.032$).

Compared with the halcinonide group, the HIFU group exhibited enhanced and statistically significant ($P = 0.039$) curative effects. Regarding changes in the different patterns, only epithelial atrophy exhibited statistically significant differences. However, the limited case numbers might not have practical significance. The statistical results of the comparisons between the 2 groups are shown in Table 4.

Table 2. Pathological results prior to and after treatment in the HIFU group.

	Prior to treatment	After treatment	P
Acanthotic pattern	33	32	0.8083
Lichenoid pattern	31	9	< 0.0001
Sclerosis pattern	29	8	< 0.0001
Chronic inflammation	46	35	0.0141
Epithelial atrophy	2	1	0.5708
Diagnosis			< 0.0001
Non-specific inflammation	9	22	
SH	22	31	
LP	2	1	
LS	29	8	

Table 3. Pathological results prior to and after treatment in the halcinonide group.

	Prior to treatment	After treatment	P
Acanthotic pattern	31	30	0.7629
Lichenoid pattern	22	8	0.0003
Sclerosis pattern	20	7	0.0002
Chronic inflammation	39	29	0.0220
Epithelial atrophy	7	4	0.2592
Diagnosis			0.0032
Non-specific inflammation	16	28	
SH	23	25	
LP	2	1	
LS	20	7	

Table 4. A comparison of the therapeutic effects between the 2 groups.

	P
Acanthotic pattern	0.7064
Lichenoid pattern	0.1614
Sclerosis pattern	0.1493
Chronic inflammation	0.1204
Epithelial atrophy	0.0376
Diagnosis	0.039

Discussion

Non-neoplastic epithelial disorders of the vulva comprise a group of inflammatory lesions, the pathogenesis of which remains unknown (12). The common types of NNEDV include squamous cell hyperplasia, lichen simplex chronicus, lichen planus and lichen sclerosus. LS is the most common form of NNEDV, and its histological changes include lichenoid patterns and dermal sclerosis (13, 14). Previous studies have demonstrated that in LS, the microvessels are reduced, leading to reduced blood flow (10, 15). Although several methods for treating NNEDV have been attempted, patients have been told that these disorders are incurable (12). Currently, corticosteroids represent the first-line treatment for NNEDV because of its anti-inflammatory effects (1, 16). However, after the treatment, the patients require long-term topical steroid use to maintain remission, which might cause well-known side effects.

Because of the thermal effects of ultrasound absorption, the HIFU treatment has been used to treat human tumors (6). Furthermore, ultrasound treatment can stimulate cell proliferation, protein synthesis and revascularization as well as accelerate tissue reconstruction and improve the microcirculation and nutrition of local tissues (17, 18). Recently, several studies have demonstrated encouraging results of HIFU in treating NNEDV (10, 17). We found that LS was significantly reduced or disappeared after the HIFU treatments. While comparing the patterns of the diseases, the lichenoid and sclerosis patterns, and chronic inflammation were also markedly reduced or disappeared. Of the 62 patients in the HIFU group, our team analyzed the clinical observations of 29 patients. The patients experienced definite therapeutic effects (19). Our histological results, which were consistent with the clinical results, confirmed the therapeutic effects of HIFU treatment in NNEDV.

Corticosteroids, which are a first-line treatment for NNEDV, clearly exhibited curative effects. In this study, we also enrolled a group of 61 patients treated with Halcinonide to serve as a control cohort. As expected, the diagnoses prior to and after treatment were significantly different. There were also prominent reductions in the lichenoid and sclerotic patterns and chronic inflammation. While comparing the effects of the 2 methods, we found that HIFU elicited better therapeutic effects with significant differences both in pathology reversal (P=0.039) and symptom improvement. Furthermore, whereas one requires a single HIFU treatment to attain such therapeutic effects, the corticosteroids must be administered for 3 months at least twice weekly. These results might better represent the compliance of the HIFU treatment. Significant therapeutic effects, good compliance, and fewer side effects of long-term steroid use make HIFU a competitive treatment.

We observed that some LS cases changed to SH in both treatment groups. Most of the SH cases that were diagnosed prior to treatment persisted (14/22 cases in the HIFU group and 18/23 in the halcinonide group). This result, which was inconsistent with previous reports (10, 17), and might be attributed to the fact that our pathologists are more sensitive to SH. The definition of SH is “an increase in the number of epithelial cells”. Thus, we diagnosed SH when the epithelium was thickened, with an increased number of acanthocytes as well as the extended epithelial ridge. Squamous cell hyperplasia is known not to have a higher risk of malignancy, which might represent a type of response to chronic stimulation. Neither of the treatments elicited prominent effects on the SH cases, which could be explained as a chronic mild response that required long-term treatment for recovery. For the LS cases that changed to SH, the patterns of lichenoid, sclerosis and chronic inflammation disappeared. Although there were still SH, the elasticity, shape and appearance of the skin recovered to some extent, and the symptoms of the patients were ameliorated by increased quality of life scores. Thus, we believed that the therapeutic effects were achieved.

Although we confirmed that HIFU treatment elicits definite effects on NNEDV over a period of months, we have not received the long-term follow up data from the patients treated with HIFU. Currently, there have not been any reports on the long-term results of HIFU. The long-term efficacies of HIFU still must be observed.

In conclusion, from a histological viewpoint of this relatively short, randomized, controlled trial, HIFU represents an effective method for treating NNEDV. Because it is a single course treatment with good compliance, it represents a promising treatment method. Long-term investigations are required to evaluate the continuing effects of HIFU.

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