

Review Article

Effects of ATP treatment on p53 protein and immune function in patients with leukoplakia vulvae

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Abstract: This paper aimed to investigating the effects of ATP treatment on p53 protein expression and immune functions of patients with leukoplakia vulvae. A total of 246 patients with leukoplakia vulvae who were admitted to the Chongqing hospital of traditional Chinese medicine were enrolled as research subjects; in which 128 cases were treated with ATP (study group) and 118 cases were conventionally treated (control group). Before and after treatment, the positive expression of p53 protein and changes in T lymphocyte subsets were detected and compared between the two groups. After treatment, the clinical efficacy in the patients was recorded, and the 6-month recurrence of disease was tracked and observed. After treatment, the scores of: pruritus degree, frequency, and duration in both groups reduced remarkably ($P<0.05$), and the scores in the study group were remarkably lower than those in the control group ($P<0.05$). After treatment, the negative expression of p53 protein in the study group increased remarkably ($P<0.05$). After treatment, the levels of CD3⁺, CD4⁺, and CD4⁺/CD8⁺ in both groups remarkably increased ($P<0.05$), and those in the study group were remarkably higher than those in the control group ($P<0.05$). After treatment, lesion severity scores in both groups reduced remarkably ($P<0.05$), and the scores in the study group were remarkably lower than those in the control group ($P<0.05$). After treatment, the clinical efficacy in the study group was remarkably better than that in the control group ($P<0.05$). Patients in both groups experienced no adverse reactions during treatment. The 6-month recurrence rate of the disease in the study group was remarkably lower than that in the control group ($P<0.05$). In conclusion, ATP treatment can treat patients with leukoplakia vulvae. It can significantly improve immune function, reduce p53 protein expression, and relieve pruritus symptoms and lesion severity, as well as lower the recurrence rate of the disease.

Keywords: ATP, leukoplakia vulvae, p53 protein, immune function

Introduction

Characterized by chronic changes in vulvar nutrition and also clinically called vulvar dystrophy [1], leukoplakia vulvae occurs both physiologically (menopause) and pathologically (lichen sclerosus and vulvar graft versus host disease) [2]. Its risk factors include spicy foods, frequent anger or tension, increased intensity of labor, vulvitis, and urethritis [3]; and its clinically common symptoms include pruritus, burning sensation, dyspareunia, and vaginal dryness [4]. Currently, the major methods for treating the disease are Chinese herbal medicine therapy, acupoint point injection therapy, electrothermal acupuncture therapy, and the combination of Chinese herbs with western medicine [5]; all of which, however, cannot completely relieve the above symptoms. Therefore, adenosine tri-

phosphate (ATP)-infrared biological effect therapeutic instruments have been applied for the clinical treatment of leukoplakia vulvae, in recent years. The optical bands of the instrument combine pulse modulation and light guidance and filtering technologies; all of which can be used to physiologically repair tissues and cells. The temperature of the bands is close to that of the human body, and biological energy released by the bands is the same as that released by ATP during hydrolysis. Thus, the bands can replace ATP molecules to provide energy for cells, activate them, and promote their normal growth and development, thereby physiologically repairing diseased tissue [6-8].

According to some studies, patients with lichen sclerosus in leukoplakia vulvae have an increased risk of squamous cell carcinoma, with

the skin of the vulva in postmenopausal women, considered as the most frequent site of attack and pruritus is considered as the most frequent symptom [9]. Tumor suppressor protein p53 plays a great role in cancer prevention. Its enhanced and active forms control the expression of target genes in cells via binding to DNA response elements (REs), further inhibiting tumor cells from growth and prevention of cancer [10].

The broad mutation spectrum of the p53 gene is the basis of tumor development and progression [11]. According to previous studies, p53 mutation has been found in more than 50% of cancers [12]. Since it can regulate angiogenesis, this gene is a crucial regulatory mediator for cell apoptosis, proliferation, and growth [13]. Leukoplakia vulvae is caused by pathological changes in cells, which cannot absorb nutrition from the body, so patients' immune function and recovery capabilities decrease. As a result, the patients are complicated with various gynecological diseases [14].

In this paper, the positive expression of p53 protein and related immune function in patients with leukoplakia vulvae were detected before and after treatment. Additionally, the clinical efficacy of treatment was recorded, and recurrence of the disease was tracked and observed; so as to evaluate the effects of ATP treatment on the protein expression and immune function in the patients.

Materials and methods

General clinical data

A total of 246 patients with leukoplakia vulvae who were treated in the Chongqing Hospital of Traditional Chinese Medicine, were enrolled; in which 128 cases received ATP treatment (the study group), with an average age of (42.17 ± 4.28) years and their average course of disease was (3.21 ± 0.45) years. The study group consisted of 56 cases of squamous hyperplasia, 30 cases of lichen sclerosus of vulva, and 42 cases of mixed malnutrition. The other 118 cases received conventional treatment (the control group), and their average age was (43.04 ± 4.56) years and their average course of disease was (3.24 ± 0.41) years. The control group consisted of 51 cases of squamous hyperplasia, 32 cases of lichen sclerosus of vulva, and 35 cases of mixed malnutrition.

Inclusion criteria: Patients who were accompanied by their family members at the time of admission; patients with complete clinical data; patients who voluntarily cooperated in the follow-up investigation; patients without acute and chronic infectious diseases. Exclusion criteria: Patients with previous mental or nervous system diseases; patients who suffered from communication disorders and could not cooperate in examinations due to dysphoria and aphasia; patients with immune and endocrine diseases; patients who had received treatment within 15 days before admission.

The patients and their family members were informed in advance of the research, and they agreed and signed a complete informed consent form. The Ethics Committee of our hospital approved this study.

Therapeutic methods

Conventional treatment: Patients in the control group were treated with an external coating of dexamethasone acetate cream (Shanghai General Pharmaceutical Co., Ltd., H31022411) twice a day. Twenty days were considered as one course of treatment, and the second course began 7 days after the first course. The patients were treated with 3 courses of treatment. Additionally, they were administrated with diethyl stilbestrol tablets (Shuguang Pharmaceutical Co., Ltd., Beijing, H11021071), once a day, with half of a tablet each time. Twenty-one days were considered as one course of treatment, and the second course began 7 days after the first course. The patients were treated for 3 courses of treatment.

Patients in the study group received irradiation therapy using the ATP-infrared biological effect therapeutic instrument (Harbin On Pine Great Medical Technology Co., Ltd., YZB/Hei0062-2013). Their external genital was irradiated from the lesion center to the outside, exceeding the lesion outer margin by 1-2 cm. The patients were irradiated with 30 W of power twice a day for 10 minutes each time. Then, their treatment intervals were continuously adjusted based on their specific symptoms, and gradually extended as the symptoms were relieved until they completely disappeared.

Outcome measures

Changes in the patients' pruritus symptom scores and skin lesion range scores were

Table 1. Comparison of general information ($\bar{x} \pm sd$)/n [%]

	Study group (n=128)	Control group (n=118)	χ^2/t	P
Average age (Years)	42.17 $\pm$ 4.28	43.04 $\pm$ 4.56	1.54	0.12
BMI (kg/m ²)	22.41 $\pm$ 2.04	22.39 $\pm$ 2.12	0.08	0.94
Average course of disease (Years)	3.21 $\pm$ 0.45	3.24 $\pm$ 0.41	0.55	0.59
Symptom classification			0.53	0.77
Squamous hyperplasia (Cases)	56 (43.75)	51 (43.22)		
Lichen sclerosus of vulva (Cases)	30 (23.44)	32 (27.12)		
Mixed malnutrition (Cases)	42 (32.81)	35 (29.66)		
Drinking			0.02	0.89
Yes	51 (39.84)	46 (38.98)		
No	77 (60.16)	72 (61.02)		
Smoking			0.03	0.86
Yes	36 (28.13)	32 (27.12)		
No	92 (71.87)	86 (72.88)		
Complications				
Hypertension	61 (47.66)	57 (48.31)	0.01	0.92
Hyperlipemia	52 (52.34)	49 (51.69)	0.02	0.89

observed. Before and after treatment, an enzyme-linked immunosorbent assay (ELISA) was adopted for detecting p53 expression (human p53 ELISA kits, Shanghai Guduo Biotechnology Co., Ltd., GD-S0197-J) in leukoplakia vulvae, with the steps strictly carried out based on the kit's instruction. Before and after treatment, venous blood from the elbow (3 mL each) was drawn from the patients and then placed into heparin anticoagulant tubes. A flow cytometer (Beijing Image Trading Co., Ltd., Item No.: AMG0002051) was adopted for detecting CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ (kits, Shanghai Youyu Biotechnology Co., Ltd., Item No.: JM596). After treatment, the clinical efficacy of treatment in the patients was recorded, and those with marked efficacy and relieved symptoms were followed up for 6 months to observe recurrence status.

Judgment criteria for skin lesion ranges were from no skin lesion to lesions visible to naked eyes (0-3 points). Judgment criteria for pruritus degree were no pruritus, occasional pruritus, paroxysmal pruritus, and severe pruritus (0-3 points). Judgment criteria for pruritus frequency were no pruritus, 1-2 times/day, 3-5 times/day, and >5 times/day (0-3 points). Judgment criteria for pruritus duration were divided into 4 grades (0-3 points): no pruritus, 1-30 minutes each time, 0.5-1 hour each time, and longer than 1 hour each time.

Statistical methods

In this study, all statistical results were analyzed by SPSS 20.0 (International Business Machines Corporation, New York, USA). GraphPad Prism 7 (GraphPad Software, Inc., San Diego CA, USA) was adopted for illustration of figures. Count data were expressed as [n (%)], and compared between groups by a chi-square test. Measurement data were expressed as ($\bar{x} \pm s$), and compared between two groups by a t test. The difference was statistically significant when $P < 0.05$.

Results

Comparison of general information

The general clinical data of the patients were compared between the study and control groups (**Table 1**). The differences were not significant between the two groups in terms of their average age, body mass index (BMI), average course of disease, symptom classification, drinking, smoking, and complications ($P > 0.05$).

Comparison of pruritus symptom scores before and after treatment

The pruritus symptom scores before and after treatment were compared between the study and control groups (**Figure 1**). Before treatment, the differences were not significant in the

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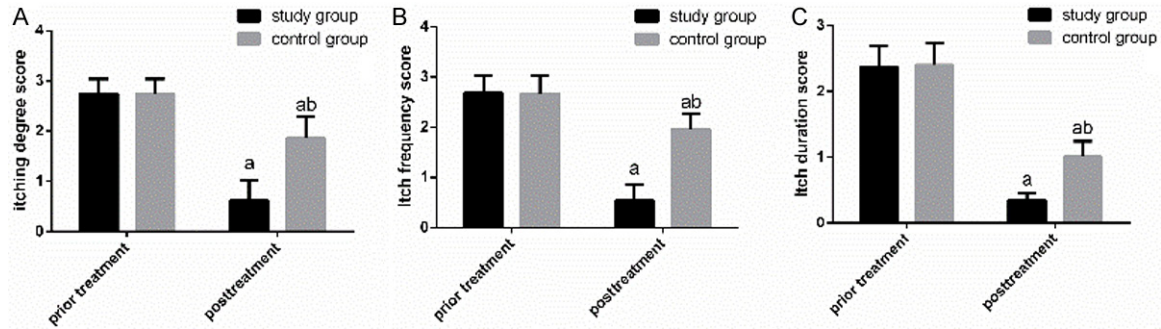


Figure 1. Comparison of pruritus symptom scores before and after treatment. A. The comparison of pruritus degree scores between the two groups after treatment. B. The comparison of pruritus frequency scores between the two groups after treatment. C. The comparison of pruritus duration scores between the two groups after treatment. Before treatment, the differences were not significant in the three scores between the two groups. After treatment, the scores in both groups remarkably reduced, and those were remarkably lower in the study group ($P < 0.05$). Note: ^a $P < 0.05$, the comparison between before and after treatment in the same group. ^b $P < 0.05$, the comparison between two groups after treatment.

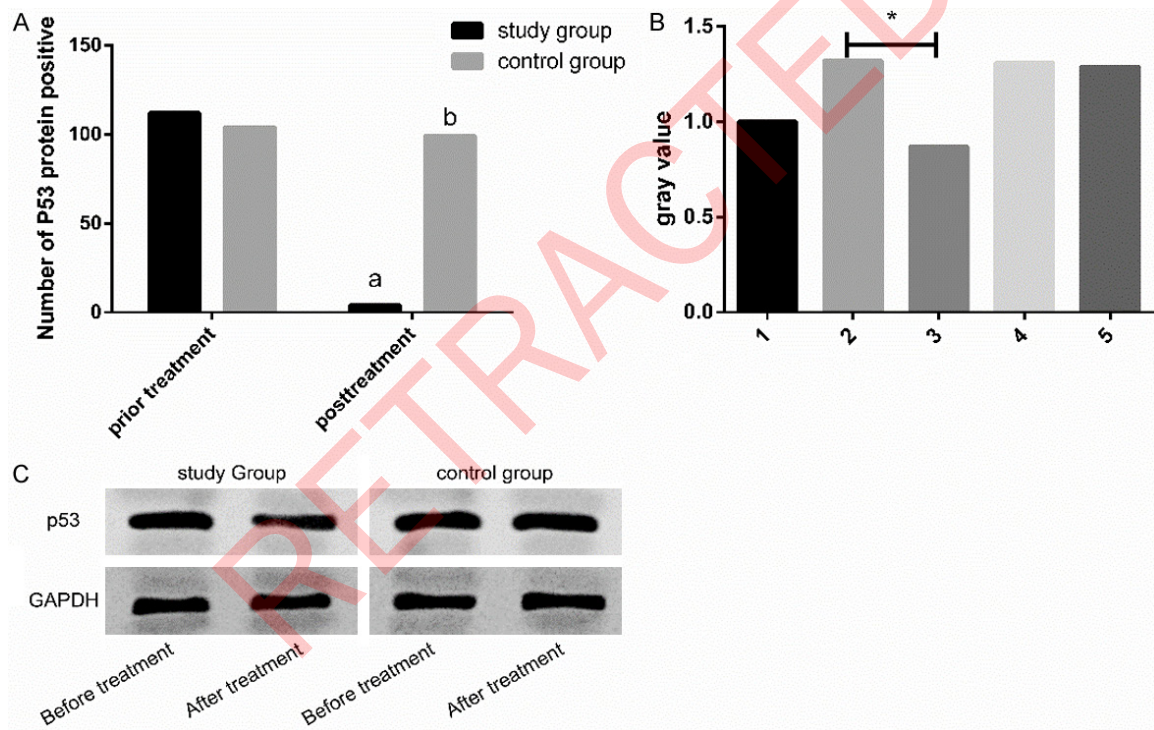


Figure 2. The comparison of the number of p53 positive expression cases before and after treatment. A. Before treatment, the difference was not significant in p53 expression between the study and control groups. After treatment, the number of p53 positive expression cases in the study group remarkably reduced, while the number of p53 expression cases in the control group was not remarkably different from that before treatment. B. The comparison of gray value before and after treatment. In the study group, p53 expression was significantly different between before and after treatment. C. WB images of p53 protein in both groups. Note: ^a $P < 0.05$, the comparison between before and after treatment in the same group. ^b $P < 0.05$, the comparison between two groups after treatment. 1 indicates the gray value of the internal reference protein. 2 indicates the gray value of p53 protein in the study group before treatment. 3 indicates the gray value of p53 protein in the study group after treatment. 4 indicates the gray value of p53 protein in the control group before treatment. 5 indicates the gray value of p53 protein in the control group after treatment.

three scores between the two groups ($P > 0.05$). After treatment, the scores in both groups

remarkably reduced ($P < 0.05$), and they were remarkably lower in the study group ($P < 0.05$).

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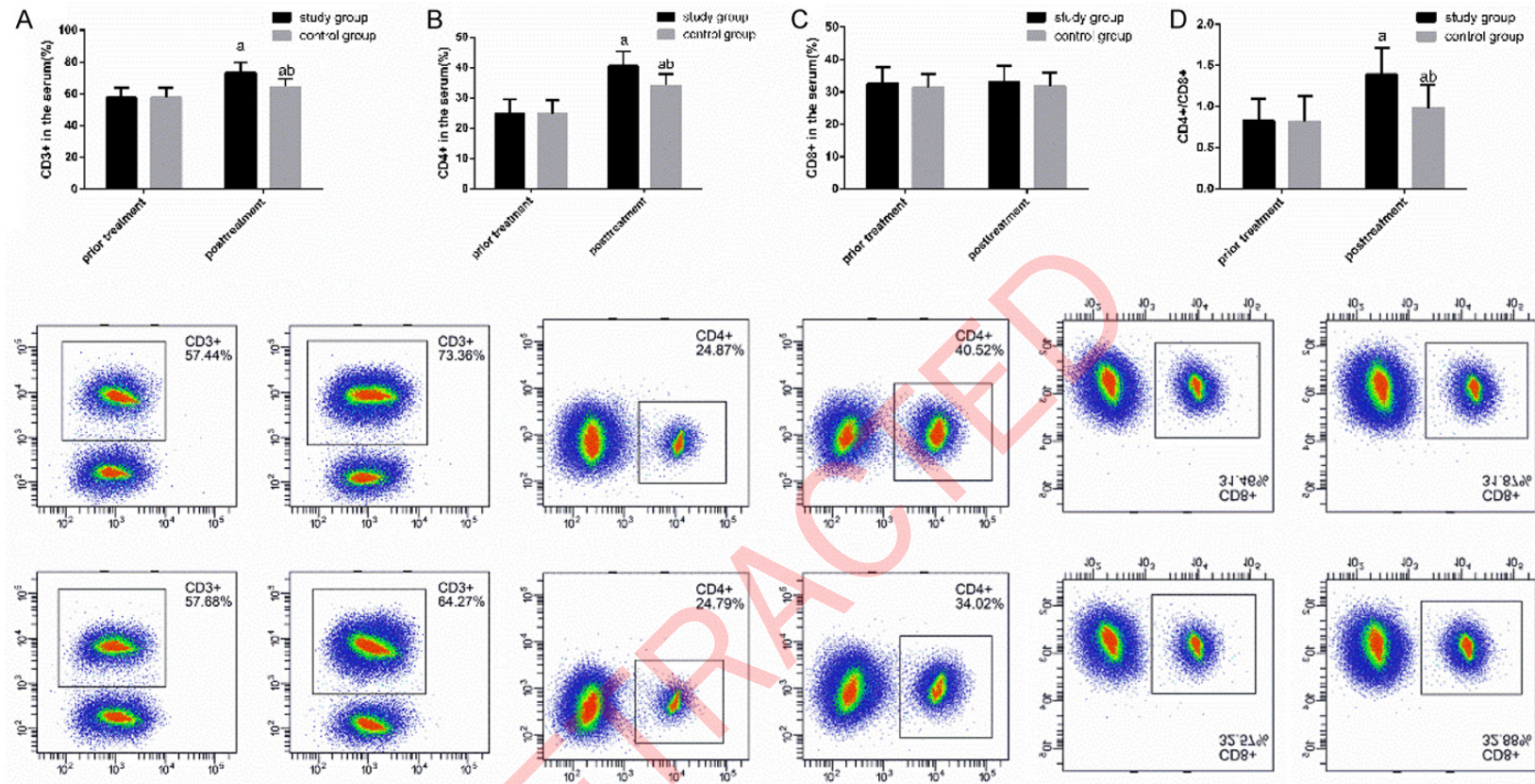


Figure 3. Comparison of indices of immune functions before and after treatment. After treatment, serum CD3⁺, CD4⁺, and CD4⁺/CD8⁺ remarkably rose, and the indices were remarkably higher in the study group, while serum CD8⁺ did not remarkably change in the two groups. Note: ^aP<0.05, the comparison between before and after treatment in the same group. ^bP<0.05, the comparison between two groups after treatment.

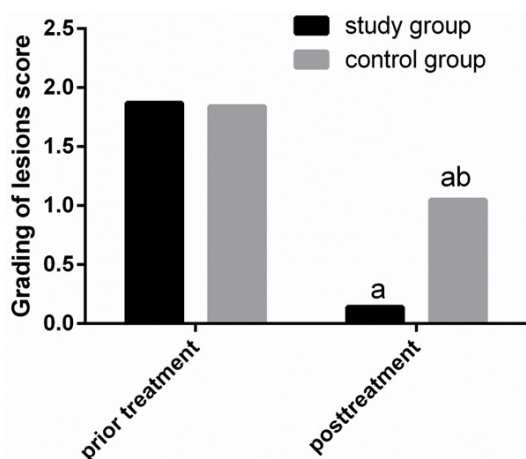


Figure 4. Comparison of lesion severity scores before and after treatment. Before treatment, the differences were not significant in lesion severity scores between the study and control groups. After treatment, the scores in both groups remarkably reduced, and the scores were remarkably lower in the study group. Note: ^a $P < 0.05$, the comparison between before and after treatment in the same group. ^b $P < 0.05$, the comparison between two groups after treatment.

Comparison of positive p53 expression before and after treatment

The positive expression of p53 protein before and after treatment was compared between the study and control groups (**Figure 2**). Before treatment, the difference was not significant in p53 expression between the study group (16 negative cases and 112 positive cases) and the control group (14 negative cases and 104 positive cases) ($P > 0.05$). After treatment, the number of p53 positive expression cases in the study group (4 positive cases) remarkably reduced ($P < 0.05$), while the number of p53 expression cases in the control group (19 negative cases and 99 positive cases) was not remarkably different from that before treatment ($P > 0.05$).

Comparison of indices of immune functions before and after treatment

Indices of immune function before and after treatment were compared between the study and control groups (**Figure 3**). Before treatment, the differences were not significant in CD3⁺, CD4⁺, CD8⁺, and CD4⁺/CD8⁺ levels between the two groups ($P > 0.05$). After treatment, serum CD8⁺ did not remarkably change in the two groups ($P > 0.05$), while serum CD3⁺,

CD4⁺, and CD4⁺/CD8⁺ remarkably rose ($P < 0.05$), and the indices were significantly higher in the study group ($P < 0.05$).

Comparison of lesion severity scores before and after treatment

Lesion severity scores before and after treatment were compared between the study and control groups (**Figure 4**). Before treatment, the differences were not significant in the scores between the two groups ($P > 0.05$). After treatment, the scores in both groups remarkably reduced ($P < 0.05$), and the scores were remarkably lower in the study group ($P < 0.05$).

Comparison of clinical efficacy after treatment

The post-treatment clinical efficacy was compared between the study and control groups (**Table 2**). After treatment, the efficacy was remarkably better in the study group ($P < 0.05$).

Comparison of adverse reactions and follow-up recurrence

No adverse reactions occurred in either group of patients during treatment. After treatment, the 6-month recurrence of 204 patients with marked efficacy and relieved symptoms was followed up. All 204 of them successfully returned for follow up visits (**Table 3**). After treatment, the 6-month recurrence rate was remarkably lower in the study group ($P < 0.05$).

Discussion

Because of the interaction between electromagnetic waves and physiological organisms, the ATP-infrared biological effect therapeutic instrument with a non-thermal biological effect can physiologically repair tissues and cells under non-invasive conditions [15]. This solves the difficult problems of physiologically repairing the natural healing function of women's external genital tract tissues and cells as well as that of treating diseases of the mucosal immune system. Patients with leukoplakia vulvae have reduced immune functions [16]. Cellular immunity is the main force in eliminating intracellular pathogen infection and also in anti-tumor immunity [17]. T lymphocytes, not as single cells but as a multifunctional cell population, are mainly responsible for this immunity [18]. They are also the main parameters reflect-

Table 2. Comparison of clinical efficacy after treatment

	Markedly effective	Effective	Ineffective	Total effective rate
Study group (n=128)	78 (60.94)	43 (33.59)	7 (5.47)	121 (94.53)
Control group (n=118)	31 (26.27)	52 (44.08)	35 (29.65)	83 (70.35)
χ^2	-	-	-	25.38
P	-	-	-	0.00

Table 3. Comparison of recurrence after treatment [n (%)]

	Recurrent pruritus vulvae	No symptoms	Recurrence rate
Study group (n=121)	6 (4.96)	115 (95.04)	4.96%
Control group (n=83)	21 (25.30)	62 (74.70)	25.30%
χ^2	-	-	17.74
P	-	-	0.00

ing the body's immunoregulation capability, among which CD3⁺, CD4⁺, and CD8⁺ exert a pivotal function in cellular immunity, and the CD4⁺/CD8⁺ ratio reflects the body's immune function the most [19, 20]. CD3⁺ represents total T cells, CD4⁺ represents helper T cell subsets, and CD8⁺ represents inhibitory cytotoxic T cell subsets [21].

According to some studies, reduction in estrogen secretion during female menopause results in the thinning of skin of vulva as well as the loss of skin elasticity and moisture, which is prone to cause itching. Besides, vulvar squamous hyperplasia and lichen sclerosus also aggravate pruritus vulvae [22]. Lichen sclerosus, the most common disease of female genital skin, often causes pruritus, pain, and other sensory symptoms [23]. In our study, after treatment, the three pruritus symptom scores in both groups remarkably reduced. This indicates that both conventional treatment and ATP light irradiation treatment can relieve the pruritus symptoms caused by leukoplakia vulvae. The three scores were remarkably lower in the study group, which reveals that ATP light irradiation treatment is better than conventional treatment in relieving the pruritus symptoms. Moreover, lesion severity scores in both groups reduced remarkably after treatment. This is because the two therapeutic methods can repair the patients' skin lesion tissues, and possibly because the patients' pruritus degree reduced. The scores were remarkably lower in the study group, indicating that ATP light irradiation treatment can better repair the patients' skin lesion and reduce their infection rate. p53

expression is related to various cancers [24-26], and this protein is negatively expressed in normal human bodies. Lichen sclerosus of vulva and high-risk human papilloma viruses are two main pathogenic factors of vulvar squamous cell carcinoma [27]. In our study, after treatment, the number of p53 positive

expression cases in the study group remarkably reduced, while the number of p53 expression cases in the control group was not remarkably different from that before treatment. These findings demonstrate that ATP light irradiation treatment can better restore the patients' health and reduce their cancer probability compared with conventional treatment. Some studies have shown that p53 is not only a crucial protein for cell defense against tumorigenesis, but also involved in a host antiviral defense proteins [28]. The immune functions of the body can be understood via detecting the T lymphocyte subsets of patients [29-31]. In our study, after treatment, serum CD3⁺, CD4⁺, and CD4⁺/CD8⁺ in both groups remarkably rose, while the indices were remarkably higher in the study group. This shows that ATP light irradiation treatment can better restore the immune functions of patients with leukoplakia vulvae. Patients in the study and control groups experienced no adverse reactions during treatment. After treatment, the clinical efficacy in the study group was 94.53%, which was remarkably better than 70.35% in the control group. The 6-month recurrence after treatment was tracked, and the recurrence rate in the study group was 4.96%, which was remarkably lower than 25.3% in the control group. These findings reveal that compared with conventional treatment, ATP light irradiation can treat leukoplakia vulvae, with no adverse reactions and a lower recurrence rate.

In this paper, before and after treatment, positive p53 expression and changes in immune function were detected and compared between

the two groups. Additionally, the clinical efficacy of treatment in the patients was recorded, and the recurrence of leukoplakia vulvae was tracked and observed, so as to comprehensively discuss the effects of ATP intervention on the p53 protein expression and immune function of patients with leukoplakia vulvae. However, this paper still has limitations. The specific mechanism of action of ATP on the patients was not studied, and there were many factors affecting the patients. Additionally, the cure and recurrence of the patients in different environments can be specifically analyzed in future research. Therefore, more perfect experimental analyses will be performed as soon as possible to obtain the best experimental results.

In summary, ATP intervention can treat patients with leukoplakia vulvae. It can significantly improve their immune functions, reduce their p53 expression, and relieve their pruritus symptoms and lesion severity, as well as lower the recurrence rate of the disease. Therefore, ATP treatment has a high clinical value.

Disclosure of conflict of interest

None.

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