https://doi.org/10.31925/farmacia.2023.5.12 **ORIGINAL ARTICLE**

EFFECT OF CARBON DIOXIDE LASER COMBINED WITH ANTI-HPV BIOLOGICAL PROTEIN DRESSING ON HPV LOAD IN PATIENTS WITH *CONDYLOMA ACUMINATUM*

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Abstract

The aim of this study was to investigate the therapeutic effect of a carbon dioxide (CO_2) laser combined with an anti-human papillomavirus (HPV) biological protein dressing on patients with *condyloma acuminata* (CA). In this study, male CA patients in the hospital were selected as research subjects and enrolled into the control group (30 cases, CO_2 laser treatment) and the observation group (30 cases, CO_2 laser combined with anti-HPV biological protein dressing treatment). The viral DNA load of the two groups was determined and compared before treatment, after treatment and at 18 months of follow-up. The treatment response rate and relapse rate of the two groups were also compared. After 18 months, both control and observation viral DNA loads were significantly lower ($p < 0.05$). After treatment and 18 months of follow-up, the viral DNA loads in the observation group were significantly lower than the viral DNA loads in the control group ($p < 0.05$). The efficacy rate (93.33%) of the observation group was significantly higher than that of the control group (63.33%), the long-term recurrence rate (13.33%) was significantly lower than that of the control group (43.33%), and the adverse reaction rate (23.33%) of the observation group was significantly lower than that of the control group (56.67%) (all $p < 0.05$). Therefore, a CO_2 laser combined with an anti-HPV biologic protein dressing can significantly reduce the HPV load in CA patients, improving the cure rate and reducing the recurrence rate.

Rezumat

Scopul acestui studiu a fost de a investiga efectul terapeutic al unui laser cu dioxid de carbon (CO_2) combinat cu terapie proteică locală anti-HPV (virusul papiloma uman) la pacienții cu condilom acuminat (CA). În acest studiu, pacienții de sex masculin cu CA, spitalizați, au fost împărțiți în 2 grupuri: grupul de control (30 de cazuri, tratament cu laser CO_2) și grupul de observație (30 de cazuri, tratament cu laser CO_2 combinat cu pansament proteic biologic anti-HPV). Încărcătura de ADN viral a celor două grupuri a fost determinată și comparată înainte de tratament, imediat după tratament și la 18 luni de la finalizarea tratamentului. De asemenea, au fost comparate ratele de răspuns la tratament și de recidivă în cele două grupuri. După 18 luni, încărcătura cu ADN-ul viral în grupul de control, cât și în grupul de observație au fost semnificativ mai mici ($p < 0,05$). Imediat după tratament și la 18 luni de la finalizarea tratamentului, încărcăturile de ADN viral din grupul de observație au fost semnificativ mai mici decât încărcăturile de ADN viral din grupul de control ($p < 0,05$). Rata de eficacitate (93,33%) a grupului de observație a fost semnificativ mai mare decât cea a grupului de control (63,33%), rata de recurență pe termen lung (13,33%) a fost semnificativ mai mică decât cea a grupului de control (43,33%), iar rata reacțiilor adverse (23,33%) a grupului de observație a fost semnificativ mai mică decât cea a grupului de control (56,67%) ($p < 0,05$). Prin urmare, un laser CO_2 combinat cu o terapie proteică locală anti-HPV poate reduce semnificativ încărcătura HPV la pacienții cu CA, îmbunătățind rata de vindecare și reducând rata de recurență.

Keywords: CO_2 laser, anti-human papillomavirus biologic protein dressing, *condyloma acuminata*, virus load

Introduction

Condyloma acuminata (CA) is a prevalent sexually transmitted infection resulting from the infection of human papillomavirus (HPV), making it the second most frequently encountered sexually transmitted disease globally [1, 2]. CA occurs mainly in young and sexually active groups, as the sexual intercourse is the main mode of transmission. Early sexual behaviour and multiple sexual partners are considered high-risk factors for HPV infection [3-5]. HPV is widespread in nature and humans are the only host for HPV. HPV enters the body through open sores in the skin or mucous membranes. Studies have shown that about 70 to 80 percent of women are infected with HPV at least once in their lives. There are currently more than 200 HPV subtypes, which are divided into low-risk and high-risk types based on their association with benign and malignant lesions. CA is usually caused by a low-risk HPV infection, of which HPV-6 and HPV-11 are the most common. The clinical manifestations of CA patients are flesh-coloured papules or cauliflower-like lesions, and the lesion sites in male patients are most commonly the coronal sulcus of the penis and the foreskin [6–10]. There is a

pressing need to enhance awareness and understanding pertaining to sexual health, with a concurrent emphasis on individuals actively mitigating the presence of detrimental sexual connections within their own lives. Currently, there are a variety of treatments for CA, including surgery, immunotherapy and local treatment [11, 12]. CO_2 laser is one of the most widely used methods in recent years and has been used to treat various types of warts since the 1980s. The CO_2 laser mainly involves two mechanisms, with the beam focus acting as a scalpel to remove the wart and the beam defocus vaporising and removing the wart through high-temperature infrared irradiation of the lesion tissue. This method is less costly and invasive, but because HPV is concentrated in the dermis of warts and the infectious rash is small, it is not easy to detect. Therefore, clinical studies have confirmed that HPV cannot be completely eliminated after CO_2 laser treatment.

In addition, other studies have shown that simple and repeated use of lasers can easily leave scars on patients, resulting in delayed wound healing and a relatively high recurrence rate [13–15]. CA recurrence not only has a serious impact on the physical and mental health of patients, but also increases the risk of developing cancer and places an enormous economic and psychological burden on patients and their families. Consequently, the reduction of recurrence rates among patients with CA following CO_2 laser treatment has emerged as a prominent topic of clinical study both nationally and internationally. The utilisation of an anti-HPV biological protein dressing has been shown to potentially contribute to the eradication of HPV. This dressing mostly consists of carbomer and JB protein [16]. The JB protein can bind to the HPV capsid protein, change its conformation, inactivate it and competitively inhibit virus binding to host cells. Carbomers can absorb and encapsulate the inactivated virus, eliminate it from the body, help repair local damage and promote wound healing. Therefore, the anti-HPV biological protein dressing can specifically prevent HPV virus invasion and block HPV infection. The combination of an anti-HPV biological protein dressing and CO_2 laser therapy in the treatment of CA patients reduces scarring. The multiplex real-time HPV test is a new method of HPV detection that can simultaneously determine the HPV type and viral load of 21 HPV types.

Existing literature has demonstrated that the determination and prognosis of lesions can be influenced by the load of HPV. Additionally, the likelihood of infection in individuals following contact with CA patients is primarily contingent upon the viral load [17, 18]. The effectiveness of the method can be assessed by measuring the viral load before and after treatment. Therefore, it is of great importance to investigate the effect of CO_2 laser combined with anti-HPV biological protein dressing on HPV load in patients with CA.

The primary objective of the study was to compare the viral DNA loads of the two groups at different stages: before treatment, after treatment and following an 18-month follow-up period. This comparative analysis aimed to assess the treatment response rate and recurrence rate in both groups, ultimately determining the therapeutic efficacy of CO_2 laser treatment in conjunction with anti-HPV biological protein supplements for CA patients.

Materials and Methods

General information

The study included 60 CA patients who were hospitalised between December 2019 and December 2020 at the First Hospital of Changsha, Hunan, China. The inclusion criteria encompassed patients who met the diagnostic criteria for CA, were verified through HPV testing, and had not undergone prior systematic treatment. Furthermore, only male patients with lesions in the prepuce and glans were eligible.

Exclusion criteria for the study were established to exclude patients with underlying conditions such as diabetes mellitus, hypertension, liver and kidney dysfunction and other diseases. Additionally, patients with malignant tumours, acquired immunodeficiency syndrome (AIDS), syphilis, or those who had used immunosuppressive agents were not included. Patients with incomplete or missing clinical data were also excluded from the study.

The selected patients were then randomly assigned into two groups: the control group, consisting of 30 patients, and the observation group, also comprising 30 patients. In the control group, patients received treatment with a CO_2 laser, while in the observation group, they received the same CO_2 laser treatment, augmented with anti-HPV biological protein supplements. Informed consent was obtained from the patients or their family members, and the research protocol was officially approved by the ethics committee of The First Hospital of Changsha, China.

Therapeutic methods

General clinical data such as age, disease course, number of skin lesions and pathogenic factors were analysed. The control group (n = 30) was treated with a CO_2 laser (DM-300; Qingdao Aoyouding Information Technology Co., Ltd., China). First, the patient's wart site and surrounding skin were disinfected, followed by local anaesthesia with 2% lidocaine (Shanghai Chaohui Pharmaceutical Co., Ltd., Shanghai, China). The CO_2 laser therapy device was then used to burn the lesion site vertically until the warts were completely removed. After treatment, Baiduobang (Mupirocin Ointment) (GlaxoSmithKline Pharmaceuticals Ltd., Tianjin, China) was applied and the area was wrapped with gauze (Jilin Furan Medical Technology Co., Ltd., Jilin, China). Patients required daily sitz baths and

topical fusidic acid ointment (Aumei Pharmaceutical, Hong Kong, China) to prevent infection.

The observation group (n = 30) was treated with a CO_2 laser combined with an anti-HPV biological protein dressing (Specifications: 3 g *per* device; Taiyuan Jinbo Biomedical Technology Co., Ltd., Taiyuan, China). After removal of the warts with CO_2 laser, the anti-HPV biological protein dressing (3 g *per* branch) was applied 3 times a day for continuous treatment for 14 days, with an application area of more than 1 mm on the wound skin.

Laboratory testing

Before treatment, a sterile brush was moistened with normal saline, and a sufficient number of exudated cells were taken from the patient's lesions and placed in sterile tubes filled with normal saline. Nucleic Acid Extraction: DNA extraction and purification of the samples were performed using the BeyoMag™ Magnetic Bead-based PCR/DNA Purification Kit (Biyuntian Biotechnology, China). A proteinase K solution with a final concentration of 40 mg/mL in 1 mL of deionized water was prepared. The patient's lesion cells were lysed using RIPA cell lysis buffer (MedChemExpress (MCE), USA). Subsequently, 500 μL of the virus lysis buffer (VL) was added to a sterile centrifuge tube, followed by the addition of the prepared proteinase K and 25 μL of virus magnetic beads. To this mixture, 200 μL of cell homogenate was added, and the solution was thoroughly mixed before incubating in a water bath at 55°C for 20 minutes. The centrifuge tube was then placed on a magnetic stand for magnetic separation, and after 90 seconds, the supernatant was removed using a pipette. Next, buffer A for washing was added to the centrifuge tube, mixed well and placed on the magnetic stand. After 90 seconds, the supernatant was removed using a pipette. Subsequently, 700 μL of buffer B for washing was added to the centrifuge tube, left to stand for 10 seconds and then the supernatant was removed using a pipette. To elute the nucleic acid, 80 μL of elution buffer was added to the tube, followed by incubation in a water bath at 56°C for 5 minutes with intermittent vortexing (3 - 4 times) to release the nucleic acid from the magnetic beads. The centrifuge tube was placed back on the magnetic stand, and after 60 seconds, the liquid containing the extracted sample DNA was collected. After the extraction of viral DNA, HPV genotypes were determined by the HPV 21 typing quantitative monitoring system (Jiangsu Shuoshi Biotechnology Co., Ltd., China), and the samples were tested for the 18 high-risk HPV subtypes specified by the China Food and Drug Administration (CFDA), as well as the 3 common low-risk subtypes, HPV 6, 11 and 81. The HPV viral load in the same number of cells was calculated by quantifying the number of cells shed by single-copy gene detection.

Evaluation indicators

Both groups were followed up for 18 months after treatment. The treatment effects of the two groups were compared according to the clinical manifestations of the patients, and the evaluation criteria were divided into effective, ineffective and recurrent. The specific criteria are shown in Table I. Viral loads were compared before treatment, after treatment and after 18 months of follow-up.

Table I

Efficacy evaluation criteria

Curative effect	Standard
Effective	Most or all warts disappeared after 6 months of follow-up
Ineffective	Most warts remained after 6 months of follow-up
Long-term recurrence	During the follow-up period of 18 months, no CA patients were exposed, but new warts appeared in the original lesion site or adjacent sites, and the PCR test was positive

$$Treatment\ response\ rate = \frac{\text{The number of effective persons}}{\text{The total number of persons in the group}} * 100\% \quad (1)$$

$$Treatment\ inefficiency = \frac{\text{Ineffective number}}{\text{The total number of persons in the group}} * 100\% \quad (2)$$

$$Treatment\ recurrence\ rate = \frac{\text{The number of recurrences}}{\text{The total number of persons in the group}} * 100\% \quad (3)$$

$$Incidence\ of\ adverse\ reactions = \frac{\text{The number of adverse reactions}}{\text{The total number of persons in the group}} * 100\% \quad (4)$$

Statistical methods

All data were statistically analysed using SPSS 26.0 (IBM, USA). The measurement data were analysed by *t*-test, and the count data were analysed by chi-square test. A $p < 0.05$ indicates a significant difference and statistical significance.

Results and Discussion

General data of the patients included in the study

In the control group, the average age was 34 ± 7.7 years (range, 22 - 48 years). The course of the disease ranged from 1 to 11 months, with an average of 6.32 ± 2.01 months. The number of lesions was 4.77 ± 1.04. The number of people infected by sexual contact was 21, and the number of people infected by nonsexual

contact was 9. The age of patients in the observation group ranged from 22 to 50 years old, with an average age of 35.7 ± 8.31 years old. The course of the disease ranged from 1 to 12 months, with an average of 6.84 ± 3.23 months. The number of lesions was 4.1 ± 1.06. There were 23 people infected by sexual contact and 7 infected by nonsexual contact. There was no significant difference in age, disease duration, number of skin lesions, pathogenic factors, or other general data between the two groups ($p > 0.05$), which was comparable (Figure 1).

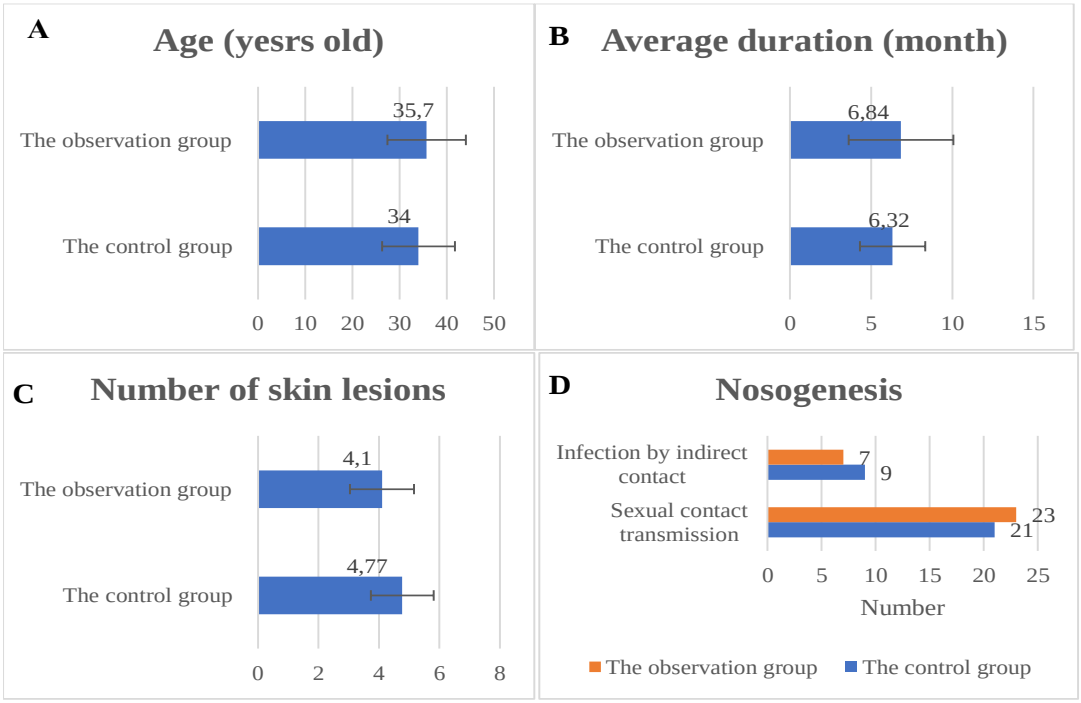

Figure 1.

Comparison of general data between the two groups. (A) represents the average age of patients in the two groups; (B) represents the average course of disease in the two groups; (C) is the number of skin lesions in the two groups; and (D) represents the pathogenic factors of patients in the two groups

Comparison of efficacy

The results showed that in the control group, the effective rate was 63.33% (19/30), the ineffective rate was 36.67% (11/30) and the long-term recurrence rate was 43.33% (13/30). In the observation group, the effective rate was 93.33% (28/30), the ineffective rate was 6.67% (2/30), and the long-term recurrence rate was 13.33% (4/30). The treatment response rate of the observation group was higher than that of the control group, and the long-term recurrence rate was lower than that of the control group ($p < 0.05$) (Figure 2).

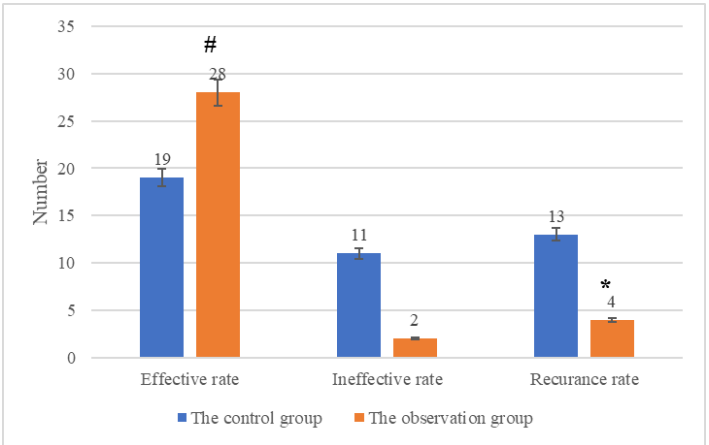

Figure 2.

Comparison of the treatment effects between the two groups
($p < 0.05$ compared with the control group; $p < 0.01$ compared with the control group)

Comparison of adverse reactions between the two groups

The results showed that the adverse reactions after treatment in the control group included pain 10% (3/30), pruritus 6.67% (2/30), infection 6.67% (2/30), redness 13.33% (4/30) and scarring 20% (6/30) and the overall incidence was 56.67%. The post-treatment adverse events in the observation group were pain 3.33% (1/30), pruritus 6.67% (2/30), infection 3.33% (1/30), redness 6.67% (2/30) and scarring 3.33% (1/30), and the overall incidence was 23.33%. The adverse reaction rate of the observation group was lower than that of the control group ($p < 0.05$). The adverse reactions in both groups could be controlled after drug treatment, which did not affect the follow-up study (Figure 3).

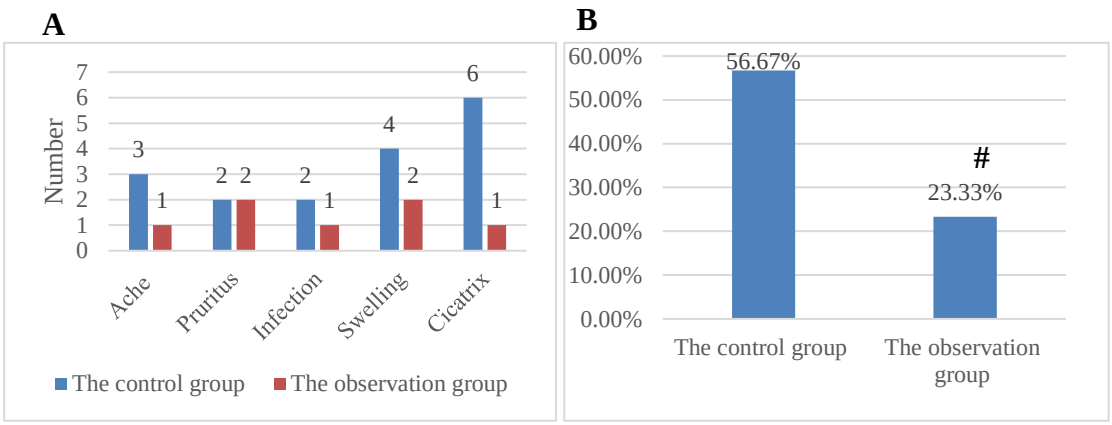

Figure 3.

Comparison of adverse reactions between the two groups. (A) represents the number of adverse reactions in the two groups; (B) represents the comparison of the overall incidence of adverse reactions between the two groups (# $p < 0.05$ compared with the control group)

HPV typing

The results showed that the most common types in both groups were HPV-6 and 11. The HPV types contained in the control group included HPV 6, 11, 16 (high-risk), 34, 45 (high-risk), 51 (high-risk), 53 and 56 (high-risk). The HPV types in the observation group included HPV 6, 11, 16 (high risk), 31 (high risk), 51 (high risk), 52 (high risk), 53 and 56 (high risk). There was no significant difference in HPV typing between the control group and the observation group ($p > 0.05$), which was comparable (Figure 4).

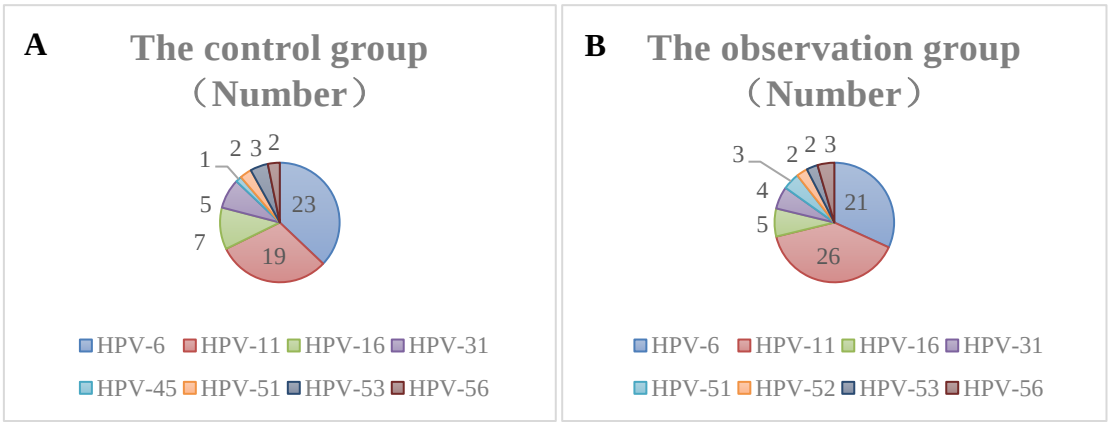

Figure 4.

HPV typing in the two groups. (A) represents HPV typing in the control group; (B) represents HPV typing in the observation group

Comparison of viral DNA load

The results showed that the viral load in the control group was $3.25 \pm 1.23 \times 10^7$ copies before treatment, $2.31 \pm 1.46 \times 10^5$ copies after treatment and $1.19 \pm 3.51 \times 10^7$ copies after 18 months of follow-up. The viral load of the observation group was $(3.76 \pm 1.57) \times 10^7$ copies before treatment, $(2.86 \pm 2.51) \times 10^4$ copies after treatment and $(1.75 \pm 3.12) \times 10^4$ copies after 18 months of follow-up. Compared with the pre-treatment level, the viral DNA load of the control group and the observation group was significantly lower after treatment and after 18 months of follow-up,

and the differences were significant ($p < 0.05$). The observation group's viral DNA load was significantly lower than the control group's after treatment and 18 months ($p < 0.05$) (Figure 5).

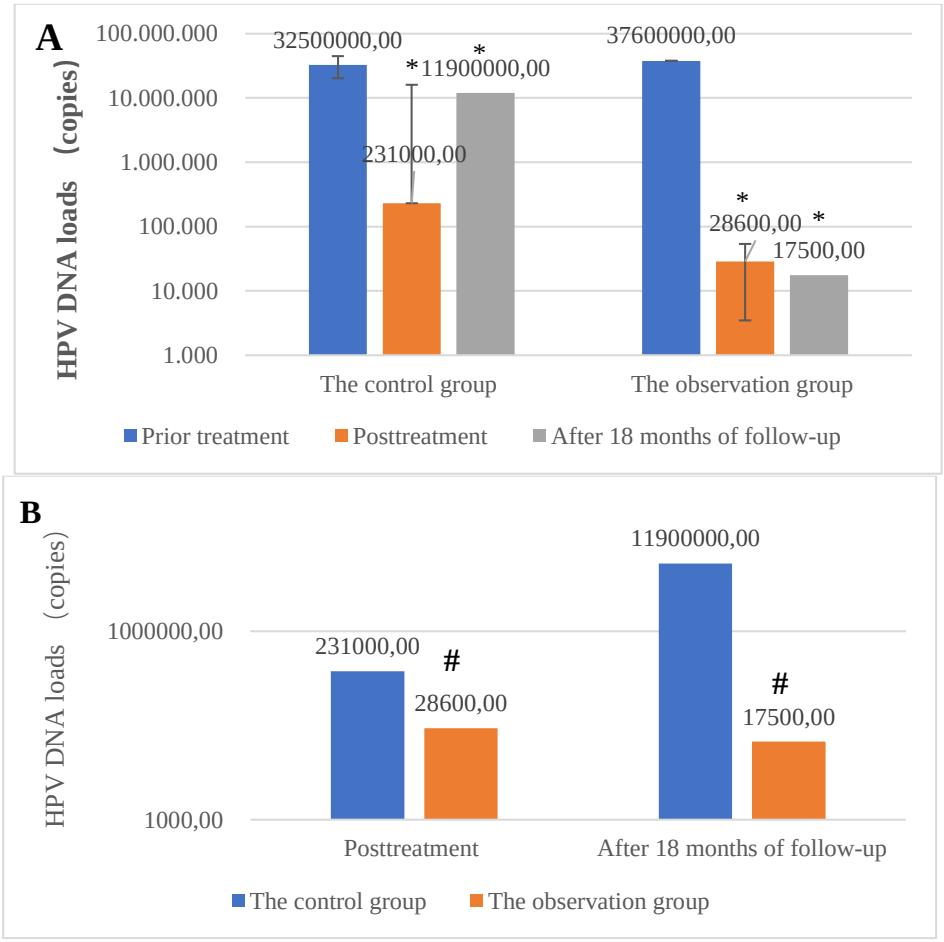

Figure 5.

Comparison of HPV DNA load between the two groups. (A) represents the viral load of the control group after treatment and 18 months of follow-up compared with that before treatment and the comparison of viral load in the observation group after treatment and 18 months of follow-up with that before treatment; (B) represents the comparison of viral load between the observation group and the control group after treatment and the comparison of viral load between the observation group and the control group after 18 months of follow-up (* $p < 0.05$ compared with the level prior treatment in the same group; # $p < 0.05$ compared with the control group at the same time point)

CA is an infectious disease that often occurs in the external genital and perianal areas, mainly in young and sexually active groups. In China, the incidence of CA is increasing every year. After one-time contact with CA patients, about 60% of normal people are likely to be infected, and the infection rate is related to the HPV load, disease stage and immune function of CA patients [19]. Currently, there are many clinical treatment methods for CA, including physical therapy, topical medications and systematic drug therapy. These methods are mainly based on local removal of warts, and patients tend to relapse after being treated. The recurrence rate of patients may be related to incomplete treatment, immunodeficiency and repeated infections. The clinical management of CA patients has become a research hotspot because CA recurrence greatly increases the possibility of carcinogenesis. CO_2 laser therapy is widely used in clinical practice [20]. Although it can remove warts quickly, it has a high recurrence rate and easily causes scarring, which reduces patients' quality of life. Anti-HPV biological protein dressings can specifically prevent HPV virus invasion and block HPV infection. HPV load can determine the development and prognosis of lesions and infection after contact with CA patients is largely dependent on viral load. Therefore, it is of great importance to investigate the effect of CO_2 laser combined with anti-HPV biological protein dressing on HPV load in CA patients for clinical treatment.

Male patients with CA in the hospital were selected as research subjects and divided into a control group (30 cases) and an observation group (30 cases) according to the random number method. The control group was treated with a CO_2 laser, and the observation

group was treated with a CO_2 laser combined with an anti-HPV biological protein dressing. The viral DNA load of the two groups was determined by multiple real-time HPV. The viral DNA loads of the two groups were compared before treatment, after treatment and after 18 months of follow-up, and the treatment response rate and recurrence rate were compared between the two groups to investigate the effect of CO_2 laser combined with anti-HPV biological protein dressing on HPV load in patients with CA. The results showed that the response rate of the observation group (93.33%) was higher than that of the control group (63.33%), and the long-term recurrence rate (13.33%) was significantly lower than that of the control group (43.33%). The adverse reaction rate of the observation group (23.33%) was lower than that of the control group (56.67%), and the difference was significant ($p < 0.05$). This indicates that the CO_2 laser was effective in treating CA patients, but the recurrence rate was high, which may be because this method is not effective for latent and subclinical infectious viruses. Recurrence in CA patients is closely related to latent HPV infection [21]. The application of an anti-HPV biological protein dressing using a CO_2 laser can significantly improve the effect of treatment. CO_2 laser treatment causes local skin damage. The use of anti-HPV biological protein dressings can promote wound healing, improve patient recovery and reduce the incidence of adverse reactions. In addition, anti-HPV biological protein dressings containing carbomer and JB proteins prevent the virus from binding to host cells, significantly reducing the recurrence rate of CA patients. The results showed that the viral DNA load of patients in the control and observation groups was significantly reduced after treatment and 18 months of follow-up compared to before treatment, and the differences were substantial ($p < 0.05$). The viral DNA load of the observation group was significantly lower than that of the control group after treatment and 18 months of follow-up. This suggests that both CO_2 laser therapy and the combination of CO_2 laser therapy and an anti-HPV biologic protein dressing can reduce patients' viral load. Studies have shown that HPV load can determine the development and prognosis of lesions, and reducing viral load can improve the cure rate of patients and reduce the recurrence rate after treatment. This is of great importance for CA patients. Some studies have found that real-time detection of the viral load before and after treatment and appropriate extension of the treatment period according to the patient's viral load can improve the cure rate of patients, and some can even achieve complete clearance [22]. Therefore, for some patients with complex HPV who do not become negative on clinical treatment, a personalised cycle may be considered to reduce the patient's viral load.

Conclusions

In this study, male CA patients in the hospital were selected as research subjects and divided into the control group (30 cases) and the observation group (30 cases) according to the random number method. The control group was treated with CO_2 laser, and the observation group was treated with CO_2 laser combined with an anti-HPV biological protein dressing. The effect of the two groups on the viral load of CA patients was compared. The results showed that the viral DNA load of the two groups was significantly reduced after treatment and 18 months of follow-up compared to before treatment, and the viral DNA load of the observation group was significantly lower than that of the control group after treatment and 18 months of follow-up. However, there are still some shortcomings in this research, which may cause some errors in the results due to the small number of research samples. In conclusion, CO_2 laser combined with anti-HPV biological protein dressing can significantly reduce HPV load, improve the cure rate and reduce the recurrence rate of patients, which is worthy of clinical application.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Zayko MO, Velilla RE, Shurbaji MS, *Condyloma Acuminata* Presenting as Isolated Papillary Lesions in the Prostatic Urethra. *Am J Case Rep*., 2018; 19: 1522-1525.
2. Boda D, Docea AO, Calina D, Ilie MA, Caruntu C, Zurac S, Neagu M, Constantin C, Branisteanu DE, Voiculescu V, Mamoulakis C, Tzanakakis G, Spandidos DA, Drakoulis N, Tsatsakis AM, Human papilloma virus: Apprehending the link with carcinogenesis and unveiling new research avenues (Review). *Int J Oncol*., 2018; 52(3): 637-655.
3. Brummeisl W, Lausenmeyer E, Weber F, Bründl J, Fritsche HM, Burger M, Denzinger S, Urethrale *Condylomata acuminata* [*Urethral condylomata acuminata*]. *Urologe A.*, 2015; 54(3): 378-384.
4. Zhang R, Chen M, Yu L, Jin Z, Anticancer activity of diphenhydramine against pancreatic cancer by stimulating cell cycle arrest, apoptosis, and modulation of PI3K/Akt/mTOR pathway. *Farmacia*, 2021; 69(5): 967-973.
5. Sharifi-Rad J, Quispe C, Rahavian A, Pereira Carneiro JN, Rocha JE, Alves Borges Leal AL, Bezerra Morais Braga MF, Melo Coutinho HD, Ansari Djafari A, Alarcón-Zapata P, Martorell M, Antika G, Tumer TB, Cruz-Martins N, Helon P, Paprocka P, Koch W, Docea AO, Calina D, Bioactive Compounds as Potential Agents for Sexually Transmitted Diseases

Management: A Review to Explore Molecular Mechanisms of Action. *Front Pharmacol*., 2021; 12: 674682.
6. Thanasas I, Lavranos G, Gkogkou P, Paraskevis D, Understanding of Young Adolescents About HPV Infection: How Health Education Can Improve Vaccination Rate. *J Cancer Educ*., 2020; 35(5): 850-859.
7. Țuculeanu G, Barbu EC, Lazăr M, Chițu-Tișu CE, Olariu CM, Bojincă M, Tilișcan C, Aramă ȘS, Aramă V, ION DA, Reduced bone mineral density in young, non-cirrhotic patients with chronic viral hepatitis. *Farmacia*, 2022; 70(2): 279-286.
8. Schneider A, Pathogenesis of genital HPV infection. *Genitourin Med*., 1993; 69(3): 165-173.
9. Kirschner B, Junge J, Holl K, Rosenlund M, Collas de Souza S, Quint W, Molijn A, Jenkins D, Schledermann D, HPV genotypes in invasive cervical cancer in Danish women. *Acta Obstet Gynecol Scand*., 2013; 92(9): 1023-1031.
10. Chidobem I, Tian F, Ogbuokiri E, Mgbodile F, Mgbodile C, Jokar TO, Shah MA, Pierre-Louis F, Trends in HPV and HPV Vaccine Awareness among Gay and Bisexual Males in the U.S. *Vaccines (Basel)*, 2022; 10(4): 604.
11. Mistrangelo M, Dal Conte I, Volpatto S, DI Benedetto G, Testa V, Currado F, Morino M, Current treatments for anal condylomata acuminata. *Minerva Chir*., 2018; 73(1): 100-106.
12. Jablonska S, Traditional therapies for the treatment of condylomata acuminata (genital warts). *Australas J Dermatol*., 1998; 39(Suppl 1): S2-4.
13. Padilla-Ailhaud A, Carbon dioxide laser vaporization of condyloma acuminata. *J Low Genit Tract Dis*., 2006; 10(4): 238-241.
14. Kryger-Baggesen N, Falck Larsen J, Hjortkjaer Pedersen P, CO_2-laser treatment of condylomata acuminata. *Acta Obstet Gynecol Scand*., 1984; 63(4): 341-343.
15. Huang J, Zeng Q, Zuo C, Yang S, Xiang Y, Lu J, Kang J, Tan L, Yu X, Xi C, Huang J, Kang L, Fan F, Chen J,. The combination of CO2 laser vaporation and photodynamic therapy in treatment of *Condylomata acuminata*. *Photodiagnosis Photodyn Ther*., 2014; 11(2): 130-133.
16. Zhang X, Fu J, Wang K, Wang J, Determination of β-lactoglobulin in Anti-HPV Biological Protein Dressing by Amino Acid Assay. *Zhongguo Yi Liao Qi Xie Za Zhi*, 2021; 45(5): 551-554, (available in Chinese).
17. Hu Z, Liu L, Zhang W, Liu H, Li J, Jiang L, Zeng K, Dynamics of HPV viral loads reflect the treatment effect of photodynamic therapy in genital warts. *Photodiagnosis Photodyn Ther*., 2018; 21: 86-90.
18. Chen L, Dong B, Zhang Q, Mao X, Lin W, Ruan G, Kang Y, Sun P, HR-HPV viral load quality detection provide more accurate prediction for residual lesions after treatment: a prospective cohort study in patients with high-grade squamous lesions or worse. *Med Oncol*., 2020; 37(5): 37.
19. Spano JP, Marcelin AG, Carcelin G, HPV and cancer. *Bull Cancer*, 2005; 92(1): 59-64, (available in French).
20. Hanna R, Parker S, The advantages of carbon dioxide laser applications in paediatric oral surgery. A prospective cohort study. *Lasers Med Sci*., 2016; 31(8): 1527-1536.
21. Song PF, Chen JY, He Q, Wang J, Xu J, Pathogenicity of high risk HPV infection in pseudocondyloma of vulvae and its carcinogenicity in inducing cervical lesions. *Eur Rev Med Pharmacol Sci*., 2018; 22(6): 1541-1548.
22. Ao C, Xie J, Wang L, Li S, Li J, Jiang L, Liu H, Zeng K, 5-aminolevulinic acid photodynamic therapy for anal canal condyloma acuminatum: A series of 19 cases and literature review. *Photodiagnosis Photodyn Ther*., 2018; 23: 230-234.