



Human papillomavirus DNA detection-guided ALA-photodynamic therapy for anogenital condyloma acuminata: A report of two cases

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ABSTRACT

5-aminolevulinic acid (ALA) mediated photodynamic therapy (PDT) is a highly effective, safe, and promising treatment for human papillomavirus (HPV)-induced anogenital condyloma acuminata (CA). Specific HPV subtypes are responsible for the pathogenesis of CA and dynamic changes in viral loads may reflect the efficiency of PDT. Here we present two cases of anogenital CA successfully treated by ALA-PDT under the guidance of HPV DNA detection including genotype identification and viral load measurement, which provided clues for unexpected intra-anal infection. Both of these male patients were free of detectable HPV at the end of the treatment and no recurrence was observed after 1 year. We conclude that the HPV DNA detection can accurately guide ALA-PDT, indicate unnoticed lesions, as well as improve and evaluate the efficiency.

1. Introduction

Anogenital condyloma acuminata (CA) is one of the most common, sexually transmitted diseases in the sexually active population, nearly 90% of which are induced by the human papillomavirus (HPV) types 6 and 11 [1]. All the conventional treatments have not been definitively demonstrated to be effective in eliminating HPV infection and preventing CA from recurring [2].

As a non-invasive therapy, 5-aminolevulinic acid-mediated photodynamic therapy (ALA-PDT) has grown in popularity in CA treatment, mainly due to high efficiency in eliminating HPV and preventing recurrence [3]. 5-ALA is a biological precursor in the heme biosynthetic pathway, which leads to the accumulation of endogenous photosensitizer protoporphyrin IX (PpIX) in HPV-infected cells, inducing apoptosis after light irradiation [4,5]. The easy accessibility of light exposure and the simplicity of topical use of photosensitizers, which can be performed on some inaccessible sites for the conventional treatments, make it more suitable for intra-anogenital condyloma.

Specific HPV subtypes are responsible for the pathogenesis of CA [1]. Multiple HPV infections predispose more warts, larger warts, and a longer duration of the disease [6], which may need more rounds of PDT to achieve infection clearance [7]. Studies have shown that the viral load of HPV is associated with disease progression and high viral loads resulting from active viral replication may promote viral persistence

[8]. Dynamic monitoring of HPV viral loads can more objectively demonstrate the effectiveness and guide the treatment of PDT [9]. HPV genotype identification and viral load measurement could accurately determine the infection state of CA patients and indicate the insufficient course or incomplete therapeutic range.

2. Case 1

This case is about a 52-year-old male with repeated recurrence of perianal CA for one year after CO₂ laser and 10-session PDT. During his monthly PDT in the local hospital, warts relapsed several times in the peri-anal area and had been moved by CO₂ laser before his first visit. No warts were observed in the examination of the anal margin but the aceto-whitening test showed a positive lesion. The HPV DNA detection, including 21 HPV types (Table 1), by polymerase chain reaction was requested before PDT, and the specimen was obtained by rubbing dry swabs over the entire surface of the peri-anal lesion. The results indicated HPV-6 infection and HPV DNA viral load of 750 copies/10⁴ cells. The patient denied having anal sexual behaviors before and rejected anoscopy.

ALA-PDT was carried out after signing the consent form. 20% (w/v) ALA solution (Fudan Zhangjiang Bio-Pharmaceutical Co. Ltd., Shanghai, China) was prepared (by dissolving ALA into sterile 0.9% NaCl immediately) to place it on the aceto-whitening-positive site and

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Table 1
HPV genotypes and viral loads before and after PDT.

Patient No.	HPV genotypes	HPV Viral loads (Copies/10 ⁴ cells) before treatment (peri-anal)	HPV Viral loads (Copies/10 ⁴ cells) after treatment (peri-anal)	HPV Viral loads (Copies/10 ⁴ cells) before treatment (intra-anal)	HPV Viral loads (Copies/10 ⁴ cells) after full treatment (peri-anal and intra-anal)
1	HPV6	750	1.89E + 4	2.03E + 4	0
	HPV11	0	0	0	0
	HPV81	0	0	0	0
	HPV16	0	0	0	0
	HPV18	0	0	0	0
	HPV26	0	0	0	0
	HPV31	0	0	0	0
	HPV33	0	0	0	0
	HPV35	0	0	0	0
	HPV39	0	0	0	0
	HPV45	0	0	0	0
	HPV51	0	0	0	0
	HPV52	0	0	0	0
	HPV53	0	0	0	0
	HPV56	0	0	0	0
	HPV58	0	0	0	0
	HPV59	0	0	0	0
	HPV66	0	0	0	0
	HPV68	0	0	0	0
	HPV73	0	0	0	0
HPV82	0	0	0	0	
2	HPV6	8.91E + 6	1.43E + 6	1.68E + 5	0
	HPV11	0	0	0	0
	HPV81	0	0	0	0
	HPV16	0	0	0	0
	HPV18	0	0	0	0
	HPV26	0	0	0	0
	HPV31	0	0	0	0
	HPV33	0	0	0	0
	HPV35	0	0	0	0
	HPV39	0	0	0	0
	HPV45	0	0	0	0
	HPV51	0	0	0	0
	HPV52	0	0	0	0
	HPV53	2.39E + 4	0	0	0
	HPV56	0	0	0	0
	HPV58	0	0	0	0
	HPV59	0	0	0	0
	HPV66	0	0	0	0
	HPV68	0	0	0	0
	HPV73	0	0	0	0
HPV82	0	0	0	0	

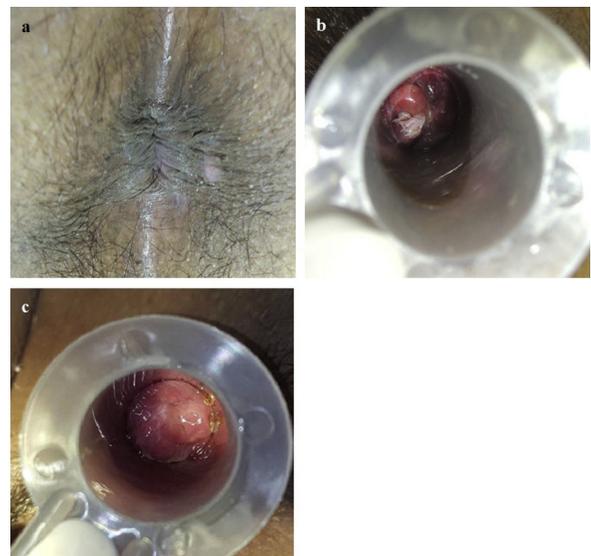


Fig. 1. Clinical findings in the patient of Case 1. (a): the peri-anal area after 4-session photodynamic therapy. (b): the verrucous lesions found in the anal canal. (c): No visible wart at the 6-month follow-up.

3. Case 2

This case is about a 34-year-old male with increased warts in the peri-anal region for 2 months. No prior treatment had been provided before his first visit. Examination of the peri-anal area showed five small-size lesions estimated about 1–5 mm, pinkish in color, with a verrucous surface. The aceto-whitening test gave positive results. HPV DNA detection was performed, which showed HPV6 and 53 infections with viral loads of 8.91E + 6 and 2.39E + 4 copies/10⁴ cells, respectively. The patient denied any anal sexual behavior before, so the anoscopy and intra-anal HPV DNA detection was not requested.

6-session PDT was done as described previously after signing the consent form. We tested HPV DNA after the sixth session, which was only HPV6 positive with a high viral load of 1.43E + 6 copies/10⁴ cells, with no visible lesion, but aceto-whitening-positive around the anal margin. An in-depth interrogation made it possible to identify the possible source of HPV. It might be an anoscopy performed by a proctologist to exclude internal hemorrhoids.

There were multiple verrucous lesions in the anal canal seen by anoscopy (Fig. 2a and b). The HPV infection was confirmed by HPV

the adjacent normal skin of 1 cm radius before covering it by a layer of opaque film for 3 h. Light irradiation of 100 J/cm² at 100 mW/cm² was applied by a cylindrical semiconductor laser fiber emitting a 635-nm laser light. Different from the long interval between two sessions in the former treatment, PDT was performed on peri-anal area weekly. HPV DNA viral load was tested again after 4 sessions, which was observed to be increased to 1.89E + 4 copies/10⁴ cells, with no visible wart but positive on the aceto-whitening test (Fig. 1a).

A detailed conversation made the patient agree to receive an anoscopy. Several verrucous lesions were found in the anal canal (Fig. 1b). The HPV infection was confirmed by HPV DNA detection showing HPV6 positive with a viral load of 2.03E + 4 copies/10⁴ cells. After removing intra-anal lesions by radiofrequency, 6 more sessions of PDT were performed both on the peri-anal area and in the anal canal. The HPV DNA detection turned out to be negative (Table 1) with no visible lesion at the end of treatment. There was no recurrence and no HPV detected at the 1-month, 6-month, and 10-month follow-ups (Fig. 1c). No recurrence was observed at the 1-year telephone follow-up.

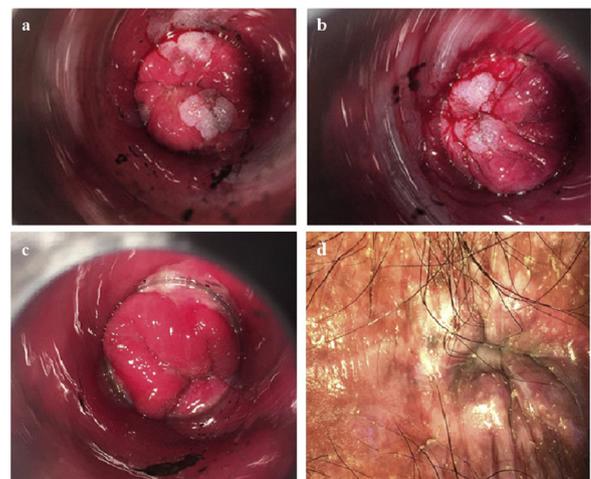


Fig. 2. Clinical findings in the patient of Case 2. (a), (b): the verrucous lesions found in the anal canal. (c), (d): No visible wart at the 3-month follow-up.

DNA detection. The result turned out to be only HPV6-positive with a viral load of $1.68E + 5$ copies/ 10^4 cells and aceto-whitening-positive. After removing intra-anal lesions by radiofrequency, 6 more sessions of PDT were performed both on the peri-anal area and in the anal canal. The HPV DNA detection was negative (Table 1) at the end of the treatment. There was no recurrence and no HPV detected at the 3-month follow-up (Fig. 2c and d). No recurrence was observed at the 6-month and 1-year telephone follow-ups.

4. Discussion

Anoscopy could be a powerful method to examine intra-anal lesions in CA patients with external anal warts, who have high-risk factors and anal sexual behavior. However, anoscopy performed by a careless person might cause mucosal injuries. Research shows HPV can be transmitted through tiny skin or mucosal breaks [10]. The wound caused by anoscopy might be an entry point for the HPV virus carried by the anoscope itself. Since there is direct contact between the anoscope and the peri-anal lesion during the examination, anoscopy may occasionally deliver the peri-anal virus into the anal canal. Therefore, anoscopy should not be performed routinely in every patient of peri-anal CA, but only in those who are at high-risk of intra-anal lesions. However, patients with a persistent positive result of HPV DNA after regular treatment should take this examination. Besides, we highly recommend that proctologist should perform an HPV DNA detection before anoscopy for a suspected HPV-infected patient with peri-anal lesions. All doctors should be more vigilant about any possible viral transmission pathway to avoid missing any suspicious lesions.

Comparing PDT with other traditional modalities, it is able to selectively and effectively target and simultaneously treat lesions simply by topical use of photosensitizers with little or no side-effects. It has evolved into a safe and effective dermatological treatment option for a variety of skin diseases such as actinic keratosis/cheilitis, superficial non-melanoma skin cancer, acne vulgaris, and verrucae [11]. As one of the non-invasive treatments for CA, PDT can completely eliminate HPV infection and carries a significantly lower recurrence rate in anogenital and urethral CA [3,12]. However, for CA, there is currently no accepted guideline for the implementation and evaluation of PDT.

HPV DNA detection including genotype identification and viral load measurement could be an indicator of the efficacy of PDT [7,9]. HPV DNA genotyping could not only identify high-risk HPV infection, which raises the awareness of oncogenesis [13], but also help to formulate an effective therapeutic strategy. Since patients with multiple-type HPV infection may need more rounds of PDT to achieve infection clearance [7], genotype identification could indicate the number of required rounds of the treatment. Besides, consistent or decreased number of genotypes might imply that there is no more exposure or reinfection after each PDT session. The dynamics of HPV DNA viral load may reflect the progress of HPV-associated diseases and predict the possibility of infection clearance [14,15]. Though there is no discernable relationship between DNA viral load and lesion causality [16], the presence of viral DNA in persistent or latent HPV infections may be associated with recurrence and refractory lesions [17]. A negative result can exclude the possibility of subclinical or latent infection at the same time. In these two cases, the HPV DNA detection provided clues of unexcepted intra-anal lesions and indicated the infection clearance after treatment. The negative results were consistent with no recurrence

during long-term follow-up. Therefore, HPV DNA detection could be a predictor of HPV infection clearance and might guide ALA-PDT, especially in the patients without visible lesions.

HPV DNA detection can be considered a powerful approach to accurately guide ALA-PDT, indicate unnoticed lesions, as well as improve and evaluate the outcome. As anoscopy may be responsible for the occurrence of intra-anal lesions, it should be performed only when highly necessary and carefully. In order to enhance the efficiency of PDT, it is important to improve awareness of any possible transmission pathway.

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Conflicts of interest

The authors have no conflict of interest to declare.

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