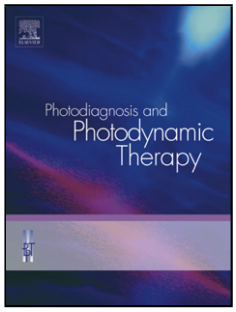

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Does HPV infection have impact on results of photodynamic treatment of vulvar lichen sclerosus?

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Abstract

Background

The association between lichen sclerosus (LS) and human papilloma virus (HPV) infections remains unclear. The co-occurrence of both pathologies possibly affecting the effectiveness of treatment and prognosis is also uncertain.

This study aimed to assess the results of photodynamic therapy (PDT) for vulvar LS and the effect of coincidence of HPV infection on the results of treatment and duration of remission.

Methods

A total of 73 patients with LS were included in the study. In each patient, 14 types of HPV were detected. PDT was performed using the PhotoDyn 501, which emits light at 630 nm wavelength and power density of 204 mW/cm². Focal lesions were exposed for 10 min once weekly for a total of 10 weeks. The complete treatment cycle was repeated after 3 months, whenever required. The biopsy was repeated after completion of treatment.

Results

The number of treatment cycles for HPV-positive and HPV-negative patients were not statistically different (cases after one or two PDT cycles). An exception was a group of patients with LS requiring three PDT cycles. Analysis of remission period considering HPV results (positive vs. negative) did not reveal a significant statistical difference. Mean remission period among HPV-negative patients was longer in comparison to remission time for those with positive HPV results (14±9 vs. 11±9 months).

Conclusions

PDT may be a promising, effective, and safe method for the treatment of LS regardless of HPV infection.

Introduction

Lichen sclerosus (LS) is an inflammatory disease of unknown etiology. The clinical course is usually chronic, and skin lesions may last for many years. LS may affect both males and females at all ages. The prevalence is 5-10 times higher in females than in males. Two peaks of the incidence rate are generally observed. The first is at puberty and the second is between 5th and 6th decades of life [1]. Incidence rate for women increases with time and reaches a peak at perimenopausal and postmenopausal periods [2,3,4].

Clinical signs manifest as white-pearl-colored lesions in the atrophied epithelium. Disease progression is associated with the induration and thickening of the pathologically changed region. Hyperkeratotic lesions (known as leukoplakia) occur around erythematous changes [3]. Hemorrhagic vesicles can occur frequently and may rupture, forming painful erosions and ulcerations that can cause secondary fungal or bacterial infections. Associated symptoms such as itching, prick like feeling, or pain decrease the quality of life. These symptoms often increase at night, causing sleep disturbances. Progression to atrophy of the vulvar folds and narrowing of vaginal entry occur in most patients. Sexual intercourse is generally not possible owing to these symptoms and the probability of the epithelial trauma [1,5].

The histological features of LS depend on the time of disease progression at the time of biopsy. Typically, there is thinning of the epithelium, with focal hyperkeratosis that is associated with edema and degeneration of the basal layer. A homogenous layer of connective tissue may be seen below the basal layer. The thickness of the connective tissue layer depends on the time of progression of the disease and is inversely proportional to the duration of the disease. The thin layer is associated with a higher treatment rate [1,3,5]. Infiltrations of T lymphocytes and Langerhans cells can be observed in the dermis. They are localized differently depending on the time of disease progression. They can be observed immediately below the basal layer in early stages or in deeper layers in more advanced stages [1]. The risk of cancerous changes within LS is approximately 4%-6.7% [6]. In recent years, the authors showed an increased incidence of vulvar cancer and precancerous cases, especially among young patients [7]. The increased incidence in this group is associated with infection of the human papilloma virus (HPV). This type of infection is confirmed in approximately 40% of vulvar cancers [7]. However, the co-occurrence of HPV infection and LS is debatable [8]. The effect of these pathologies on

each other remains unclear, as well as how the coincidence impacts the effectiveness of treatment, prognosis, and neogenesis.

The aim of this study was to assess the results of photodynamic therapy (PDT) for vulvar LS and the effect of coincidence of HPV infection on the results of treatment and duration of remission.

Materials and Methods

A total of 73 patients with LS were included in the study. The age range was 9-81 years (mean, 54.1 years). The inclusion criteria included a histological diagnosis of vulvar LS. Exclusion criteria included bacterial and fungal infections, pregnancy, previous malignant history, previous radio or chemotherapy, loss to follow-up for 2 years, and lack of patient consent.

A swab was taken from each patient for HPV-DNA using two tests (Rex Company) to determine the level of oncologic risk of the 14 types of HPV. Both tests are based on DNA fragment amplification by polymerase chain reaction. The first test was used to detect HPV types 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, and 66, and the second was used to detect HPV 6 and 11.

Vulvoscopic examination and photodynamic diagnosis were performed for all patients. A photosensitizer was used in form of gel containing 5% aminolevulinic acid (ALA) with additional substance 2% dimethyl sulfoxide (DMSO) that facilitates absorption.

This preparation was prepared under the supervision of Professor Alfreda Graczyk, a chief of the team of Laboratory of Biochemistry and Spectroscopy of the Institute of Optoelectronics of the Military University of Technology.

A photosensitizer was applied over the vulva 120 min before the photodynamic diagnosis was performed. Energy in the form of light with a wavelength of 405 nm was used to generate fluorescence. A superluminescent diode was used as the source of light (MediCom Company). Additionally, VC Camera – 101 was used to magnify the picture on a monitor 2-60 times. The computer was supplied with a program for digit registration of images and for archiving medical images miniIRIS.

Positive focal lesions, with a transitional arousal of molecules of the photosensitizer, were displayed as red color on the monitor. Negative lesions (without fluorescence) were displayed in blue color.

Target biopsy was performed depending on the above results for histopathological assessment. The biopic tissues were assessed in the Department of Pathomorphology of Brodno Hospital in Warsaw, Poland. LS was diagnosed in all patients who were then treated using PDT.

The same photosensitizer was used for treatment (containing 5% ALA with 2% DMSO). The gel was topically applied to the vulva 120 min before PDT. PDT was performed using the PhotoDyn 501 apparatus, which emits light with the wavelength of 630 nm and power density of 204 mW/cm², using an end tip of 25 cm. The following formula was used to calculate the time of light exposure considering the parameters of the apparatus:

Time of exposure (sec) = 1000 × dose (J/cm²): power density (mW/cm²)

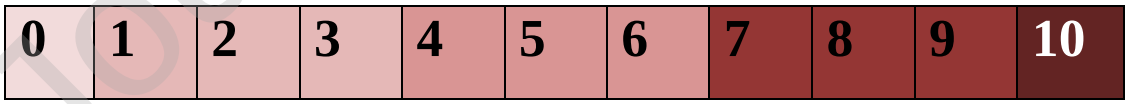
The calculation for the time exposure for a density of 120 J/cm², which is used in this study, is as follows:

120 J/cm² × 1000: 204 mW/cm² = 10 min.

Focal lesions were exposed for 10 min once weekly for a total of 10 weeks. The complete treatment cycle was repeated after 3 months, whenever required.

The highly reproducible test, known as the Numeric Rating Scale (NRS), was used to assess the symptoms reported by patients before and after PDT. The results were shown on an 11-degree scale [9].

Figure. 1. Numeric Rating Scale.



A value of 0 on this scale is used when the patient has no pain. Values of 1-3, 4-6, and 7-9 represent mild, moderate, and severe pain, respectively. A value of 10 corresponds to a very severe pain that is the worst felt, according to the subjective assessment of the patient.

Follow-up visits were planned 1,3,6,12, and 24 months after the end of PDT. Every time vulvoscopic examination and assessment of pain according to the NRS were performed. After the end of treatment, the patients underwent targeted biopsy.

The Ethics Committee of the Medical University of Warsaw approved this study (No: KB/22/2007).

Non-parametric methods were used to assess dependence and differences, as analysis of parameters in the studied groups showed deviation from normal distribution and small number of patients. The mean and standard deviation were used as parameters for normal distribution, while the median, quartiles, minimum, and maximum values were used to describe parameters that did not have a normal distribution.

Mann-Whitney U test was used to assess differences for continuous parameters with numerical values. This test checks the null hypothesis assuming that two randomly selected values belong to the same population. For each assigned value, there are ranks, and in the case of two distinct observations, tied ranks are used. Depending on the number of observations, two statistical methods were used: U when the number of observations was ≤ 20, and Z for > 20 observations. Both were comparable with the critical values for the assumed significance value.

The Wilcoxon test was used to test differences in the results of PDT. In this test, the sequence, size, and signs of differences between the results of two samples were considered during comparison of variable data pairs. After individual summary of positive and negative differences, the lower value of the sums (value of T test for a group with observation ≤ 25 and Z for a group with observations > 25) was compared to the corresponding theoretical value in the charts, which were used as reference in accepting the null hypothesis at a certain significance level.

Analysis of the proportions of persons considering certain symptoms (presence or absence) was performed using tables, for which the chi square and Pearson correlation tests were used. The exact Fisher test was used for subgroups with a low number of observations. A p value < 0.05 was assumed to be statistically significant. The STATISTICA (version 10PL) program with the academic license of Medical University of Warsaw was used for statistical assessment.

Results

Clinicopathological parameters for patients with LS are shown in Table 1. A single HPV genotype was confirmed in 14 patients, and double HPV type infection was detected in three patients. HPV 16 was the most detected strain. Low-risk HPV was found in one patient, while high-risk HPV was detected in all other patients.

Patients with vulvar diseases were considered in the statistical analysis. Patients with vulvar dysplasia were excluded from the study. Among 73 patients, six were nonparous.

Table 1. Clinicopathological parameters for patients with lichen sclerosis.

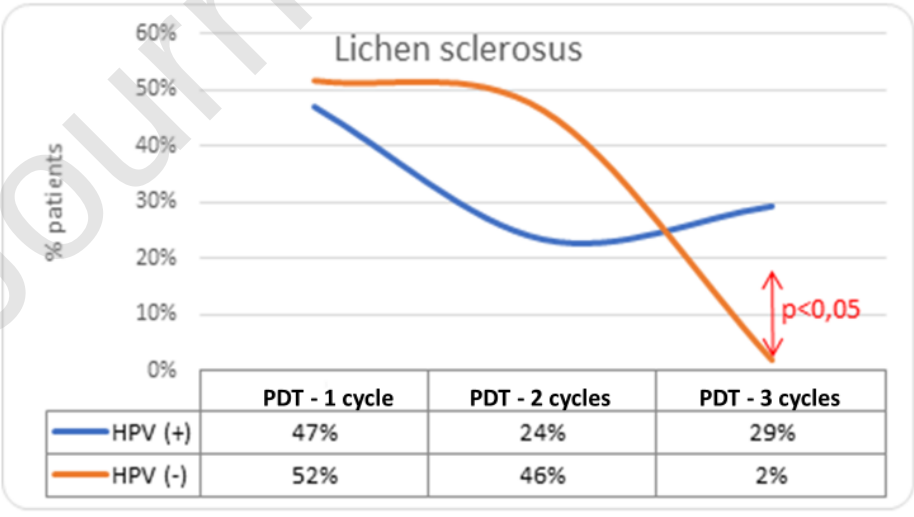
Results of HPV-DNA	Number of patients	Age in years Mean (range)
HPV(+)	17	52 (33-74)
HPV(-)	56	58,66 (9-81)

The course of treatment with PDT of 73 patients is shown in Table 2. The duration of treatment, defined as the number of cycles per LS patient, was compared considering the results of HPV-DNA. Analysis did not show an effect of HPV infection on number of treatment cycles. There was no significant statistical difference between the number of treatment cycles for HPV-positive patients with those with negative HPV (cases after one or two PDT cycles). An exception was a group of patients with LS requiring three PDT cycles. However, this group had a low number of patients. The dependence of the number of PDT cycles and results of HPV in patients with vulvar LS is shown in Table 1 and Figure 2.

Table 2. The number of used photodynamic therapy (PDT) cycles in treatment of lichen sclerosis considering results of HPV-DNA.

Histological diagnosis	Number of patients	Results of HPV	Number of patients for whom PDT was used		
			1 cycle PDT	2 cycles PDT	3 cycles PDT
Lichen sclerosus	73	HPV(+) - 17	8	4	5
		HPV(-) - 56	29	26	1
			p-ns	p-ns	p-0,002

Figure 2. The number of used photodynamic therapy (PDT) cycles for patients with lichen sclerosus (LS) considering human papilloma virus (HPV) results.



Analysis of remission period considering HPV results (positive vs. negative) did not reveal a significant statistical difference. Mean remission period among HPV-negative patients was longer compared to remission time for those with positive HPV results (14±9 vs. 11±9 months) (Table 3).

Table 3. Remission period of patients with lichen sclerosus considering human papilloma virus (HPV)-DNA.

Histological diagnosis	Number of patients	Results of HPV-DNA	Mean of remission period (p=ns)
Lichen sclerosus	17	HPV (+)	11±9 months
	56	HPV(-)	14±9 months

Remission after PDT was observed in all patients. Remission was defined as a lack of pain (scale 0 by NRS) and mild pain (scale 1-3 by NRS). Patients were subjected to a target vulvar biopsy after completion of treatment. Histological remission was observed in two cases of LS. Clinical remission of histological remission was obtained in 55 patients.

PDT was well tolerated by the patients. A total of 32 (43.8%) patients had paresthesia, which subsided after treatment. Paresthesia was noticed in the first 2-4 cycles of PDT, and it did not require intervention.

Discussion

Partial or complete remission was achieved in all patients with LS. There was no significant statistical effect of the presence or absence of HPV on the number of PDT cycles. The mean of remission of LS was shorter among patients with positive HPV compared to those with negative HPV (11±9 vs. 14±9 months). However, there was no significant statistical difference between these subgroups. Only in two LS cases with complete histological remission was confirmed after PDT. In 55 cases, there was clinical remission without histological remission.

Treatment of LS is challenging owing to the chronic nature of the disease. Treatment of choice is corticosteroids; however, 4%-10% of patients show primary resistance for treatment. A high relapse rate of up to 84% can be observed in the course of LS [10]. The second choice of treatment includes calcineurin inhibitors, retinoids, and PDT [8]. Recent evidence suggests

that PDT for LS can be effective and safe. Olejek et al. used PDT for 28 patients with LS and showed that 25 of them did not have vulvar symptoms after completion of PDT [11]. PDT was performed using a gel containing 20% ALA and laser light with an intensity of 120 J/cm^2. Hillemanns et al. used PDT for 12 patients with LS using topical gel with 20% ALA and then laser light with parameters 80 J/cm^2 and 40-70 W/cm^2 [12]. Morphine-derived analgesic therapy was used for pain relief in three patients. Reduction of symptoms was obtained in 10 out of 12 patients, and the mean time until recurrence was 6.1 months, which was half of the time in our study. Maździarz et al similar to our study, used non-laser light, with a high rate of symptoms' reduction [13]. Vano-Galvan et al. and Romero et al. effectively used PDT for the treatment of patients with LS with multiple vulvar symptoms [14,15]. In our protocol, we did not find any significant side effects.

Until now, there is a debate about the coincidence of HPV infection and LS, and their combined effect on the development of vulvar cancer is unclear [16]. There are primarily two pathological pathways for the development of vulvar cancer: squamous cell carcinoma (SCC), which is related to HPV infection (usual-type vulvar intraepithelial neoplasia [uVIN]) and the type not related to HPV infection (differentiated or simplex vulvar intraepithelial neoplasia [dVIN]) [17]. The correlation between LS and SCC as well as verrucous carcinoma is well documented. The pathways of oncogenesis in patients with LS are not clear. According to the recent hypothesis, LS can be transformed into dVIN [18,19]. It has been suggested that the progression of dVIN is associated with TP53 mutation [20]. Additionally, a recent target sequencing study revealed some other mutations such as NOTCH1 and HRASin [20,21].

Unlike dVIN, which is not related to HPV, uVIN has other histomorphological features, and the risk of progression and molecular pathomechanisms are better known. HPV infection leads to neoplasitic changes mainly through activation of viral oncoproteins E6 and E7, which leads to overexpression of cyclin-dependent kinase inhibitor protein [19].

High-risk HPV infection may initiate neoplastic changes within the LS lesion. Until now, the most important pathway for neoplastic changes and the effect on prognosis is unclear. The optimal diagnostic and therapeutic methods should be developed for assessing the effect of HPV infection on the results of LS treatment [1,19]. Physicians have already suggested that active treatment of LS may decrease the risk of malignant transformation [22]. There is recent evidence and hypothesis about the effect of high - and low-risk HPV infection on the occurrence and progression of LS [23].

Among other methods used for LS treatment, PDT seems to be effective and multidirectional. Some authors have presented effective PDT for VIN. The total histological

response is approximately 20%-67% according to various studies. The real benefit of PDT for VIN is its ability to treat multifocal lesions without loss of tissues with short healing time, minimal tissue injury, preservation of vulvar anatomy, and perfect cosmetic results. Moreover, PDT is also effective in treatment of 77.5% of cervical HPV infections [24]. Intertwining of cellular mechanisms, including necrosis, apoptosis, vascular, and more promising immunological aspects, presented PDT as a unique and comprehensive treatment modality [25].

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Highlights

1. Photodynamic therapy is effective and safe in the treatment of vulvar LS, which can co-occur with HPV infection.
2. HPV infection does not reduce effectiveness of PDT for treatment of LS.
3. HPV infection does not significantly affect the remission period.