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Research Article

A Comprehensive Approach for Preventing and Treating Squamous Intraepithelial Lesions with HPV Infection

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Abstract

Background: Human papillomavirus (HPV) is the main reason for developing cervical cancer. In 2018 about 311 thousand women died from cervical cancer, according to prognosis this condition does not tend to decrease. Presently, a lot of women are diagnosed with progressive cancer. This condition is hard to treat. Treatment Surgical methods result in uterus cervix structural and functional integrity impairment, which influence the next pregnancy and childbirth. HPV is a group of viruses that are extremely common worldwide. HPV is the most common viral infection of the reproductive tract. Photodynamic therapy (PDT) is the perspective method for treatment of cervical cancer and cervical dysplasia.

Aim: The main aim of our investigation is to find out PDT effectiveness in antiviral, antitumor treatment in squamous intraepithelial lesions and the effect of the HPV presence or absence on treatment outcomes.

Materials: The subject research is a retrospective cohort study on 489 women with acute HPV infection, SILs, ASC and invasive cancer T1. The full preoperative examination was made for each patient. This examination consisted of colposcopy with the acetic acid and Schiller's tests, PCR or Digene-test to find out HR-HPV and liquid-based cytology or histological investigation.

Results: 489 patients were treated by PDT. Previous surgical unsuccessful treatment was in 40% of cases. Full HPV elimination was 80,37% after the first PDT session. In 74,03% of patients had HPV from 1 to 6 types per woman. Presence or absence of HPV did not correlate with treatment results. The full recovery was more than 85% in each group. The effectiveness of photodynamic therapy, antitumor and antiviral effects in the treatment of precancerous and initial cervical cancer has been evaluated.

Conclusion: This study suggests that PDT can be recommended as an alternative treatment for patients with pre-malignant SILs including carcinoma *in situ* and relatively early invasive cancer of the cervix.

Keywords: *Photodynamic Therapy; Cervical Intraepithelial Neoplasia; Squamous Intraepithelial Lesions; Human Papillomavirus; Photosensitizer; HPV Infection*

Abbreviations

ASC: Atypical Squamous Cells; CIS: Cancer *In Situ*; CIN: Cervical Intraepithelial Neoplasia; CKC: Cold Knife Conization; DAMPs: Damage-Associated Molecular-Patterns Molecules; DCs: Dendritic Cells; FD: Fluorescence Diagnostics; FDA: Food and Drug Administration; HR-HPV: High-Risk Human Papillomavirus; HSIL: High-Grade Squamous Intraepithelial Lesion; LEEP: Loop Electrosurgical Excision Procedure; LSIL: Low-Grade Squamous Intraepithelial Lesion; MRI: Magnetic Resonance Imaging; NILM: Negative for Intraepithelial Lesion or Malignancy; NK: Natural Killers; PCR: Polymerase Chain Reaction; PDT: Photodynamic Therapy; PS: Photosensitizer; RFO: Reactive Forms of Oxygen; RU: Relative Units; SILs: Squamous Intraepithelial Lesions; USI: Ultrasound Investigation; UV-light: Ultraviolet Light

Introduction

This investigation gives detail on often pathology in women's health such as squamous intraepithelial lesions which are related to human papillomavirus infection. Human papillomavirus (HPV) is the main reason for developing cervical cancer. In 2018 about 311 thousand women died from cervical cancer, according to prognosis this condition does not tend to decrease [12]. Nowadays, cervical pathology and HPV infection are caused by cervical cancer in 99% cases, and HPV types 16 and 18 are in 70% of all cases [4]. Distribution of cervical cancer (6.6%) from all new cancer cases of 8.2 million, which was the fourth most common type of cancer in the world for females and the distribution of deaths (4.2 million) was the fourth (7.5%) common type of cancer too in 2018 [2]. The main idea to stop the spread of mortality is the prevention of HPV infection. Last data are recorded other types of HPV which are associated with pre-cancer pathologies, such as 31, 33, 35, 52, 58, 62 types. Primary prevention for cancer is an HPV vaccination, but this procedure cannot protect from all HPV types [9]. All these vaccines work only to prevent HPV infection. They do not treat an infection which is already there. Another type of primary prevention is using a condom in sexual life to stop spreading the etiological factor. Some resources demonstrate that condoms do not provide completely to prevent HPV infection and can be used as an additional instrument [6]. Infection can occur in a vertical way or fetal shell. Other important factors are immunosuppression, smoking, parity and oral contraceptive use. When all these factors and cofactors are induced expression HPV oncogenic proteins E6 and E7, our immune system can respond to this exposure or not.

Immune response has a key role in the development of HPV infection and complications which were caused by it. In the cases of cervical cancer, inflammation and immune response are important factors associated with the development and progression of the disease [10]. HPV viral load can be evaluated with the Digene test [7]. That is the only test which the FDA approved. Most of the existing guidelines recommend making LEEP or cold knife conization in diagnosed cases with CIN 2/3 or CIS [11]. These procedures can provide only inflammation response and not include the immune response for antiviral and antitumor effects. They have one more disadvantage, it is a relapse and recurrent infection in a cytological investigation and Digene test. Risk of malignancy is greater in 5 times in recurring lesions [5]. The rate of HPV persistence after invasive surgical procedures achieves 46% [3].

PDT is a method which includes three main components for implementation antiviral and antitumor effects [11]. Oxygen, photosensitizer and light are three fundamental things for PDT. This method is a quintessence of chemistry, biology and physics. Light is generated by a laser machine, transmitted to affected cells, tissues or organs. The photosensitizer is introduced intravenously and collected in the pathological cells, mainly in mitochondria [1]. Oxygen permeates and saturates each cell including tumor cells. The combination of these three components creates a biochemical (inflammation response) and photochemical (immune response) reactions in the cells, which activate apoptosis and necrosis. Immunological and inflammation events arise due to exposure PDT and the duration of these events over two weeks. Initial of all reactions and responses are reactive forms of oxygen, such as singlet oxygen and hydroperoxide radicals. RFO make destruction cells membranes and induce DAMPs. These molecules induce DCs and NK-cells, which activate antiviral and antitumor immune responses [1,8].

Aim of the Study

The main aim of our investigation is to find out PDT effectiveness in antiviral, antitumor treatment in squamous intraepithelial lesions and the effect of the HPV presence or absence on treatment outcomes.

Materials and Methods

1091 patients were treated by PDT from 2015 to October 2020. The subject research is a retrospective cohort study on 489 women. Inclusion criteria in the investigation were: SIL, ASC, invasive cancer T1 or acute HPV infection diagnosed, confirmed presence or absence of HPV before PDT. Each patient had no less than three controls after PDT (colposcopy + Digene test + liquid-based cytology or histological investigation). The full preoperative examination was made for each patient. This examination consisted of colposcopy with the acetic acid and Schiller's tets, PCR or Digene-test to find out HR-HPV, liquid-based cytology or histological investigation, MRI and USI pelvic organs. All patients were informed and agreed with the upcoming procedure. Chlorin E6 was used as a photosensitizer. There were intravenously injected in 1 mg per kg. The accumulation period was 3 hours in pathological tissues. Photosensitizer accumulation was controlled by fluorescent diagnostics. UV-light was generated by apparatus AFS (LLC Polaronic, Russia). Laser machine Lahta-Milon used for cervix irradiation with 662 nm wavelength. Irradiation power for the cervical canal was 400 J per cm^2 and for the external part of the cervix was 300 J per cm^2 in each field with multilocation processing. The light was delivered by cylindrical diffusor in the cervical canal. Patients with invasive cancer had a multicourse treatment protocol. Antiviral and antitumor PDT effectiveness was assessed during 1,5; 3; 6 and 24 months. PDT effectiveness estimated by Digene test and liquid-based cytology. Every patient was observed in the low dark mode for 24 hours after PS injection. The dark mode includes wearing sunglasses and illumination no more than 50 lux.

All data were estimated in a statistical package of R-programing software and Microsoft Excel. HPV types were calculated after PCR for each group. Statistical significance of differences estimated by χ^2-test with Yate's correction. Correlation between treatment results and risk factor estimated by *V* Cramer's criterion and *C* Pearson's contingency coefficient.

Results and Discussion

All patients were separated into three diagnoses groups for estimating PDT effects: first group - absolute recovery (NILM + negative Digene-test); second group - non-absolute recovery (NILM + positive Digene-test); third group - no effect (atypical squamous cells and positive Digene-test). 25 patients were with acute HPV infection (5%), 11 patients were with ASC (2%), 66 patients were with CIN1/LSIL (14%), 369 patients were with CIN2-3-CIS/HSIL (75%) and 18 patients were with invasive cancer T1N0M0 (4%) diagnoses. The range of age was from 19 to 73 y.o. The average age of patients was 34,38 y.o. High registration rate of diagnoses (49,5%) was in 26 to 35 y.o. The main reasons for this distribution might be the early onset of sexual life, disorders of innate and mucosal immunity. 196 patients had a previous surgical treatment (40,1%) such as LEEP or CKC.

In 362 patients (74,03%) were detected HPV from 1 to 6 types per woman: 16 type (75,14%); 31 type (20,44%); 33 and 52 types (19,33% and 17,68%); 18 type (15,47%); 58 type (16,57%); 35 type (13,26%); 32, 39, 44, 45, 51, 56, 57, 59, 73, 82 (less than 10%). HPV was not detected in 127 patients (25,97%).

Registration rates of HPV types in a group with acute HPV infection were for 16 type - 3,67%; 18 type - 3,57%; 31 type - 5,4%; 33 type - 4,28%; 35 type - 4,16%; 39 type - 11,76%; 45 type - 11,1%; 51 type - 7,14%; 52 type - 4,7%; 56 type - 8,33%; 58 type - 3,33% and 59 type - 9,1%.

Rates of HPV types in a group with ASC were for 16 type - 2,57%; 18 type - 7,14%; 31 type - 1,35%; 33 type - 2,86%; 35 type - 2,1%; 39 type - 11,76%; 45 type - 11,1%; 51 type - 7,14%; 52 type - 1,52%; 58 type - 9,1% and 59 type - 9,1%.

Rates of HPV types in a group with CIN1/LSIL were for 16 type - 9,92%; 18 type - 16,1%; 31 type - 14,9%; 33 type - 8,57%; 35 type - 10,4%; 39 type - 35,3%; 51 type - 35,7%; 52 type - 12,5%; 56 type - 33,33%; 58 type - 10% and 59 type - 18,2%.

Rates of HPV types in a group with CIN2/HSIL were for 16 type - 23,9%; 18 type - 23,2%; 31 type - 27%; 32 type - 100%; 33 type - 21,43%; 35 type - 29,16%; 39 type - 29,4%; 45 type - 33,33%; 51 type - 35,7%; 52 type - 21,9%; 56 type - 25%; 58 type - 28,33%; 59 type - 36,4%; 73 type - 66,67% and 82 type - 50%.

Registration rates of HPV types in a group with CIN3-Tis/HSIL were for 16 type - 54%; 18 type - 48,2%; 31 type - 37,8%; 33 type - 48,57%; 35 type - 33,33%; 39 type - 11,76%; 44 type - 100%; 45 type - 44,4%; 51 type - 21,42%; 52 type - 42,2%; 56 type - 33,33%; 58 type - 38,33%; 59 type - 27,3% and 82 type - 50%.

Rates of HPV in a group with invasive cancer T1N0M0 were for 16 type - 5,9%, 18 type - 1,8%, 31 type - 13,5%, 33 type - 14,28%, 35 type - 20,9%; 52 type - 17,2% and 58 type - 16,67%. Full HPV elimination was in 349 patients (93,65%) after the first PDT session.

Presence or absence of HPV had no correlation with treatment results. Acute HPV infection with HPV (1/14) and without HPV (0/10) had $\chi^2 = 0.043$, p = 0,835. CIN1/LSIL with HPV (10/41) and without HPV (1/14) had $\chi^2 = 0,621$, p = 0,431. CIN2/HSIL with HPV (4/79) and without HPV (1/26) had $\chi^2 = 0,084$, p = 0,772. CIN3-Tis/HSIL with HPV (17/168) and without HPV (8/66) had $\chi^2 = 0,028$, p = 0,868. T1N0M0 with HPV (2/15) and without HPV (0/1) had $\chi^2 = 1,621$, p = 0.203. Cramer's criterion and Pearson's contingency coefficient in a group with acute HPV infection was V = 0,167 and C = 0,164, in CIN1/LSIL was V = 0,146 and C = 0,144; in CIN2/HSIL was V = 0,023 and C = 0,023; in CIN3-Tis/HSIL was V = 0,025 and C = 0,025 and in invasive cancer T1N0M0 was V = 0,086 and C = 0,085 respectively. Weak correlation indicated between risk factor and treatment outcome.

In a group with acute HPV infection, 23 patients had absolute recovery (92%); 1 patient had non-absolute recovery (4%); 1 patient had no effect (4%).

In a group with ASC, 11 patients had absolute recovery (91,7%); 1 patient had non-absolute recovery (8,7%); no effect (0%).

In a group with CIN1/LSIL, 57 patients had absolute recovery (86,4%); 1 patient had non-absolute recovery (1,5%); 1 patient had no effect (1,5%) and 7 patients refused second PDT session or no data[1] (10,6%).

In a group with CIN2-3-Tis/HSIL, 342 patients had absolute recovery (92,7%); 7 patient had non-absolute recovery (1,5%); 2 patients had no effect (0,54%) and 17 patients refused second PDT session or no data* (4,6%).

In a group with invasive cancer T1N0M0, 16 patients had absolute recovery (88,9%) and 2 patients refused second PDT session or no data* (11,1%).

26 patients (5,32%) had a pregnancy after absolute recovery. Pregnancy came on average after 8 months. Pregnancy was without complications and ended in natural childbirth.

Clinical case 1: Patient with acute HPV infection.

*No data - we have no information about their control investigation after PDT.

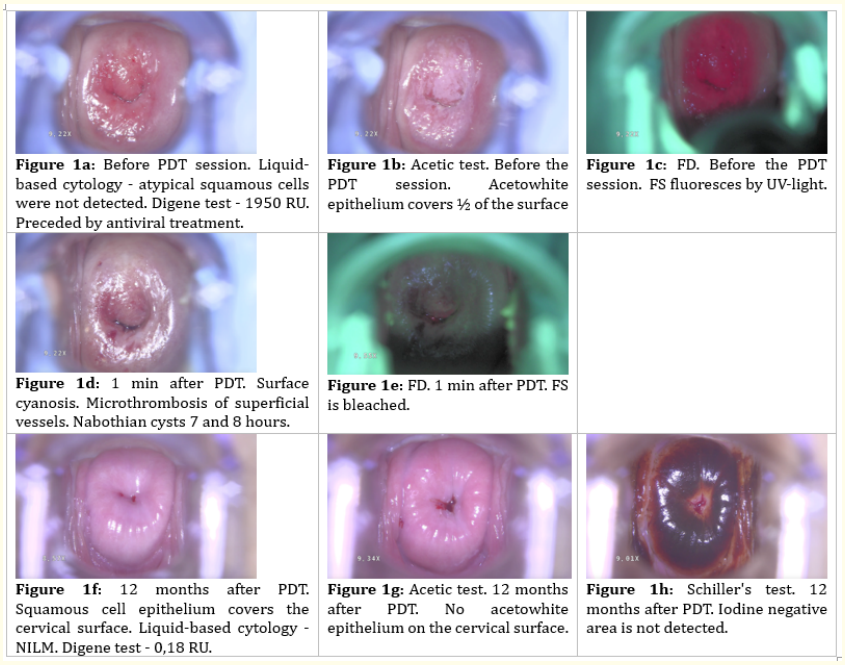

Figure 1a: Before PDT session. Liquid-based cytology - atypical squamous cells were not detected. Digene test - 1950 RU. Preceded by antiviral treatment.

Figure 1b: Acetic test. Before the PDT session. Acetowhite epithelium covers ½ of the surface

Figure 1c: FD. Before the PDT session. FS fluoresces by UV-light.

Figure 1d: 1 min after PDT. Surface cyanosis. Microthrombosis of superficial vessels. Nabothian cysts 7 and 8 hours.

Figure 1e: FD. 1 min after PDT. FS is bleached.

Figure 1f: 12 months after PDT. Squamous cell epithelium covers the cervical surface. Liquid-based cytology - NILM. Digene test - 0,18 RU.

Figure 1g: Acetic test. 12 months after PDT. No acetowhite epithelium on the cervical surface.

Figure 1h: Schiller's test. 12 months after PDT. Iodine negative area is not detected.

Clinical case 2: Patient with HSIL.

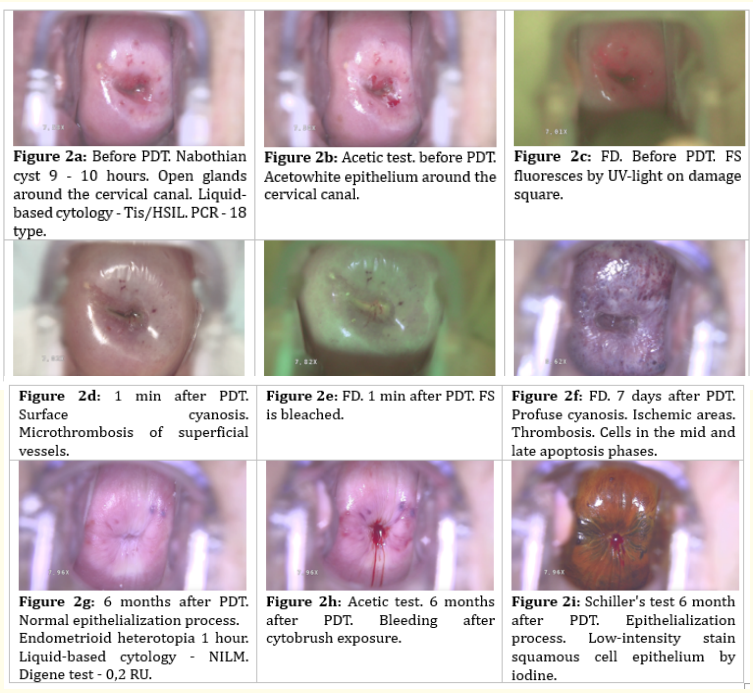

Figure 2a: Before PDT. Nabothian cyst 9 - 10 hours. Open glands around the cervical canal. Liquid-based cytology - Tis/HSIL. PCR - 18 type.

Figure 2b: Acetic test. before PDT. Acetowhite epithelium around the cervical canal.

Figure 2c: FD. Before PDT. FS fluoresces by UV-light on damage square.

Figure 2d: 1 min after PDT. Surface cyanosis. Microthrombosis of superficial vessels.

Figure 2e: FD. 1 min after PDT. FS is bleached.

Figure 2f: FD. 7 days after PDT. Profuse cyanosis. Ischemic areas. Thrombosis. Cells in the mid and late apoptosis phases.

Figure 2g: 6 months after PDT. Normal epithelialization process. Endometrioid heterotopia 1 hour. Liquid-based cytology - NILM. Digene test - 0,2 RU.

Figure 2h: Acetic test. 6 months after PDT. Bleeding after cytobrush exposure.

Figure 2i: Schiller's test 6 month after PDT. Epithelialization process. Low-intensity stain squamous cell epithelium by iodine.

Clinical case 3: Patient with invasive cancer T1N0M0.

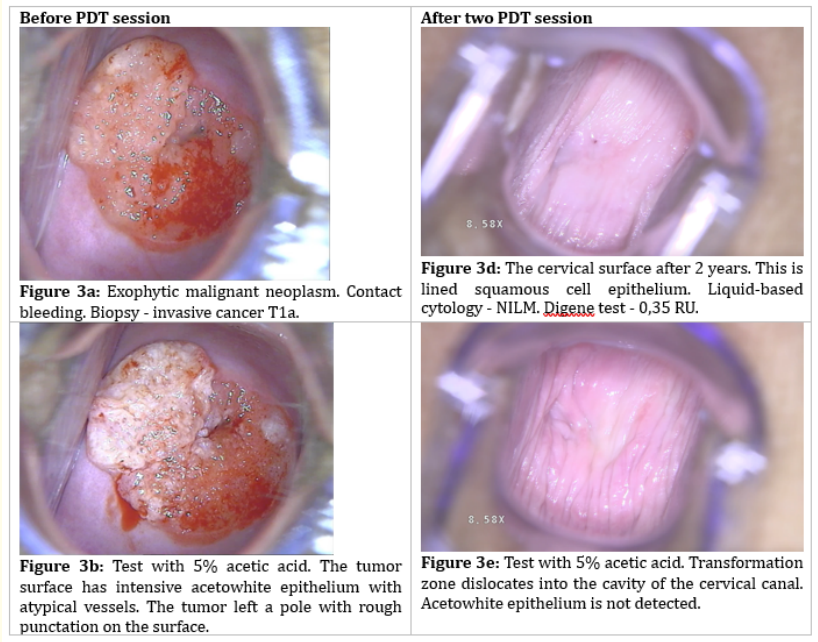

Figure 3a: Exophytic malignant neoplasm. Contact bleeding. Biopsy - invasive cancer T1a.

Figure 3d: The cervical surface after 2 years. This is lined squamous cell epithelium. Liquid-based cytology - NILM. Digene test - 0,35 RU.

Figure 3b: Test with 5% acetic acid. The tumor surface has intensive acetowhite epithelium with atypical vessels. The tumor left a pole with rough punctation on the surface.

Figure 3e: Test with 5% acetic acid. Transformation zone dislocates into the cavity of the cervical canal. Acetowhite epithelium is not detected.

Conclusion

HPV is the main factor in the development of relapses squamous intraepithelial lesions. Antiviral drugs cannot stop malignant cell transformation. An invasive surgical procedure cannot provide HPV elimination. PDT is the only treatment method, which can eliminate HPV. There is convincing data about HPV eradication after PDT, that decreases disease relapse probability. PDT is an organ-preserving treatment method of the uterus cervix pathology. It is very important for reproductive function realization. In most cases, PDT sessions were made only once, but if there were no full virus elimination, the second PDT sessions were made.

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Conflict of Interest

The authors declare no conflict of interest.

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