ORIGINAL ARTICLES

EFFECTIVENESS OF BACTERIAL BIOFILMS PHOTODYNAMIC INACTIVATION MEDIATED BY CURCUMIN EXTRACT, NANODOXYCYCLINE AND LASER DIODE

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Abstract

Biofilms have higher levels of antibiotic resistance compared to bacteria, so the alternatives are needed as therapy for diseases caused by biofilm infections. Photodynamic Therapy (PDT) has the advantage of being a safe alternative that involves molecular-level photochemical reactions. The use of different types of exogenous photosensitizers (PS) was done to compare their effectiveness. Turmeric extract containing curcumin has good effectiveness in PDT, whereas nanodoxycycline as an antibiotic has a fairly broad absorption spectrum and is effective as PS. The purpose of this study is to compare the effectiveness of photodynamic therapy on infections by *Aggregatibacter actinomycetemcomitans* causing periodontitis using exogenous organic and non-organic photosensitisers (PS). The *A. actinomycetemcomitans* biofilm had been grown on 96-well microplate for 72 hours incubation time. The samples were divided into three groups, treated with Laser diode, Laser + Turmeric Extract 0.5%, and Laser + Nanodoxycycline 0.1%. Treatment was done with a variety of exposure times: 30, 60, 90, 120, and 150 seconds. The data were analyzed using ANOVA test. The results of data analysis showed that diode laser irradiation treatment with endogenous porphyrin, diode laser with Curcumin and diode laser with nanodoxycycline produced significantly different biofilm reductions. Treatment with diode laser irradiation at various energy densities (4.15, 8.28, 12.44, 16.59, and 20.73 J/cm^2) showed no significant difference in reducing bacterial biofilm. The treatment with diode and curcumin, and the treatment with diode laser irradiation and nanodoxycyclin showed a significant difference. Diode laser irradiation of 20.73 J/cm^2 with irradiation time of 150 seconds resulted in the greatest reduction of biofilm 14.94%, diode laser irradiation + Curcumin 47.82%, and diode laser irradiation + nanodoxycyclin 53.76%. Therefore, PDT using a diode laser combined with exogenous PS extract of curcumin and nanodoxycycline is more effective to reduce bacterial biofilms.

Keywords: photodynamic inactivation, a. actinomycetemcomitans, biofilm, curcumin, extract, nanodoxycycline, laser diode.

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ЭФФЕКТИВНОСТЬ ФОТОДИНАМИЧЕСКОЙ ИНАКТИВАЦИИ БАКТЕРИАЛЬНЫХ БИОПЛЕНОК С ИСПОЛЬЗОВАНИЕМ ЭКСТРАКТА КУРКУМИНА, НАНОДОКСИЦИКЛИНА И ЛАЗЕРНОГО ДИОДА

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Резюме

Биопленки обладают более высоким уровнем устойчивости к антибиотикам по сравнению с бактериями, поэтому необходима разработка новых подходов к лечению инфекционных заболевания, вызванных бактериальными биопленками. Одним из возможных методов лечения таких заболеваний является фотодинамическая терапия (ФДТ). В качестве фотосенсибилизаторов применяли куркумин и антибиотик нанодоксициклин. Провели сравнительное изучение эффективности фотодинамической терапии инфекций, в патогенезе которых участвовали *Aggregatibacter actinomycetemcomitans*, вызывающие пародонтит, с использованием двух указанных фотосенсибилизаторов. Биопленку *A. actinomycetemcomitans* выращивали на 96-луночном микропланшете в течение 72 ч инкубации. Образцы были разделены на три группы. В первой группе проводили обработку биопленок диодным лазером, во второй – 0,5%-ым экстрактом куркумы и диодным лазером, в третьей – 0,01%-ым раствором нанодоксициклина и и диодным лазером. Время воздействия составляло 30, 60, 90, 120 и 150 сек. Полученные данные были проанализированы с использованием теста ANOVA. Результаты анализа данных показали, что эффективность воздействия на биопленки значительно отличалась в группах с облучением диодным лазером, облучением диодным лазером с куркумином и облучением диодным лазером с нанодоксициклином. Режимы облучения диодным лазером при различных плотностях энергии 4,15; 8,28; 12,44; 16,59; и 20,73 Дж/см^2 не показали существенного воздействия на бактериальную

биопленку. Облучение диодным лазером (20,73 Дж/см^2, время облучения 150 сек) привело к наибольшему уменьшению биопленки на 14,94%, облучение диодным лазером с куркумином – на 47,82%, облучение диодным лазером с нанодоксициклином – на 53,76%. Таким образом, ФДТ с использованием диодного лазера в сочетании с экзогенными фотосенсибилизаторами куркумином и нанодоксициклином показали свою эффективность в отношении бактериальных биопленок.

Ключевые слова: фотодинамическая инактивация, A. Actinomycetemcomitans, биопленка, куркумин, экстракт, нанодоксициклин, лазерный диод.

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Introduction

Indonesia has oral and dental health problems at 25.9% of its national population [1]. One of the common oral diseases that can infect almost 50% of the world's population is periodontitis [2, 3]. The number of sufferers of this disease has reportedly increased consistently over the last decade [2]. Periodontitis can be caused by bacterial activity as a parasite that exceeds the average amount in the mouth. One of them is *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*) [4, 5]. These bacteria include gram-negative bacteria that can form biofilms [6, 7, 8]. Biofilms from *A. actinomycetemcomitans* can stick to the tooth surface and form an extracellular matrix, so the level of resistance to antibiotics is high [8, 9, 10]. Therefore, effective and safe alternative treatments for periodontitis are needed, especially the disease caused by *A. actinomycetemcomitans*.

One alternative treatment for infection by bacteria developed in health is Photodynamic Therapy (PDT). This therapy utilizes Reactive Oxygen Species (ROS), which are produced through photochemical processes between light sources and chemical molecules called photosensitisers (PS) [5, 11]. The photochemical process will occur if the wavelength of the absorption of PS is matched with the light source used in PDT. PDT is claimed to be a safe therapy because it only damages the parasitic part of the target object. Therefore, as one of the main components in PDT, PS should not be toxic to healthy cells [11].

There are two types of PS used in PDT, namely endogenous PS and exogenous PS. Endogenous PS is usually an enzyme produced naturally by bacteria, such as the porphyrin in the *A. actinomycetemcomitans* [12]. Porphyrins have a maximum absorption at wavelengths of 400–450 nm [13]. Several studies suggest adding exogenous PS to maximize the effect of PDT [8, 14]. Various kinds of exogenous PS are developed for PDT, both from an organic and non-organic materials. PS from organic matter is usually obtained through extraction or isolation of a particular substance, for example, chlorophyll and curcumin [14, 15, 16]. On the other hand, the use of non-organic materials, such as antibiotics, catalysts, or dyes, was also developed as PS [17, 18, 19].

This study aims to compare the effects of PDT that occur when two types of exogenous PS are used in organic and non-organic compound. *Curcuma longa* or turmeric extracts contains curcumin. The use of curcumin as PS in PDT has a significant effect on decreasing the number of bacterial colonies [20, 21]. Several studies report the benefits of using curcumin as antimicrobial, anticarcinogenic, and anticancer agent [22]. As an ideal PS, the amount of curcumin used must be minimal but effective so that the effects arising are only due to the photodynamic process [23].

Several studies showed that it is common for photodynamic processes to utilize antibiotics as PS even though some bacteria have high resistance, such as *A. actinomycetemcomitans* [2]. Increased antibiotics resistance in bacteria can be caused by excessive use of antibiotics, thus stimulating bacteria to produce a protective form of extracellular matrix called biofilm [2], [17]. The advantage of using antibiotics as PS in PDT due to bacterial infection is an alternative function when the use of antibiotics alone is not effective enough in dealing with bacterial infections. One antibiotic with a tetracycline group that has a broad absorption spectrum is doxycycline [17]. In low doses, the doxycycline use as PS can minimize its effects as an antibiotic without causing resistance [24]. So that doxycycline can be absorbed more optimally by biofilms, doxycycline particle size is converted to nano or nanodoxycycline.

With an effective wavelength absorption range of curcumin extract at 300–500 nm and nanodoxycycline at 200–425 nm, the laser diode is appropriate for this study [17, 25]. The match between the wavelength of light and the wavelength spectrum of PS are the keys to the success of PDT [23]. When the energy received by PS is excessive, the molecule will experience excitation, and ROS is created when the excited molecule reacts with oxygen [11]. In addition, the advantage of a laser diode as a light source is that the output beam is coherent and monochromatic so that the beam diameter is smaller and more focused compared to other conventional light sources [26, 27].

Materials and Methods

Bacterial Biofilm

This study used pure isolates of *Aggregatibacter actinomycetemcomitans* ATCC 43718 obtained from the Faculty of Dentistry, Universitas Airlangga. *A. actinomycetemcomitans* biofilms were cultured on a 96-well microplate using Tryptone Soy Broth (TSB) media. Previously, bacteria were grown in a Tryptone Soy Agar (TSA) medium suspended for 6 grams/liter of yeast extract and 8 grams/liter of glucose [28]. Biofilm cultures were incubated for 72 hours at 37°C anaerobically using a candle jar [8], [28]. Optical Density (OD) of the growing biofilm was calculated using Elisa Reader.

Curcuma longa Extractions

The extraction used the maceration method. The dried turmeric rhizome has been finely immersed in 96% ethanol (C_2H_6O) solvent in a 1 gr: 10 ml [29]. Maltodextrin was added as much as 15% of the mass of the filtrate to increase the volume and final weight of the extract results and speed up the drying process [30]. Then, the ethanol in the filtrate was evaporated by using a rotary evaporator. The final filtrate, after evaporation, was then dried using an oven at 40°C. The extract powder was stored at room temperature in a dark cupboard. *Curcuma longa* as PS was a solution with sterile distilled water.

Nanodoxycycline

Doxycycline ($C_{22}H_{24}N_2O_8 \cdot H_2O$) in the form of powder was crushed for 5 hours using a mortar. Then the sample was ground using 3D High Energy Milling (HEM) to obtain nano-sized doxycycline powder. The process lasts for 2 hours using milling balls (1:20). The grinding sample is filtered using a 7.5 µm mesh. Nanodoxycycline as PS was a solution with distilled water and filtered using PTFE 0.2 µm.

Light Source

Laser Diode characterization using Jasco CT-10 Monochromator and Thorlabs PM100D Power Meter to determine the peak wavelength and intensity of the beam output. The Laser Diode specification used has a peak wavelength (403.00 ± 0.24) nm and an output beam intensity of (138.25 ± 0.01) mW/cm^2 for the beam diameter (0.20 ± 0.01) cm^2. The value of energy density laser diode can be obtained using Equation 1.

$$\text{Energy Density}\left(\text{J cm}^{-2}\right) = \text{Intensity}\left(\text{W cm}^{-2}\right) \times \text{Time exposure}\left(\text{s}\right) \quad (1)$$

Sample Treatments

The *A. actinomycetemcomitans* bacteria sampled in this study were 100 µl biofilm culture grown on 96-well microplate for 72 hours incubation time. TSB medium in each well was removed through the rinsing process using PBS with a pH of 7.4. Then exogenous PS was added in the form of 0.5% Curcumin extract or 0.1% Nanodoxycycline. After the addition of exogenous PS, biofilm culture was incubated anaerobically using a candle jar for 30 minutes, and then the laser diode exposure was performed. The sample had been treated and incubated for 24 hours. The sample taken from incubator were washed with PBS three times. The staining/coloring process uses 200 µl of crystal violet in each well for 15 minutes. The sample was rewashed using distilled sterile water three times to remove crystal violet, and then was given 100µL 33%/well glacial acetic acid (GAA) and measured using ELISA reader S/N 17539 (Bio-rad, US) on 595nm [8]. The results of the research data (OD) were converted to log CFU/ml.

The samples were divided into three treatments, Group X was treated with laser diode, Group Y was treated with laser diode+Curcumin Extract 0.5%, and Group Z was treated with laser diode+nanodoxycycline 0.1%. Treatment of laser diode with a variation of irradiation time, which were 30, 60, 90, 120, and 150 seconds with energy densities of 4.15, 8.28, 12.44, and 16.59 J/cm^2. The data were analyzed using the ANOVA test. Each treatment has a control group, namely a negative control group, a positive control group of nanodoxycycline, and a positive group of curcumin. The percentage reduction in the treated sample was compared with the control group for each treatment.

Statistical Analysis

The percentage of biofilm reduction was obtained by reducing the log CFU/ml value of the treated sample with the control group. The study results in the form of log CFU/ml were statistically analyzed by using the Statistical Package for Social Science (SPSS) version 21. The statistical test conducted was Two Way ANOVA and the Tukey test, with $p < 0.05$, so that a significant difference between the control and treatment group has a data confidence level of 95%.

Results and Discussion

Curcuma longa Extracts

Curcumin extract solution with a concentration of 0.5% is the organic exogenous PS in this study. One of the curcumin extract's contents that has the most advantage in PDT is curcumin. To know how effective curcumin extract as an exogenous PS, it is necessary to compare the absorbance between curcumin standard and curcumin extract. Fig. 1 showed a comparison of the standard absorbance of curcumin standard and curcumin extract by using the Genesys 30 Spectrophotometer.

Curcumin standard, the chemical formula [$HOC_6H_3(OCH_3)CH{=}CHCO]_2CH_2$, used in this study, was derived from Curcuma longa powder with EC number 207–280–5. Through Fig. 1, it is known that the peak absorbance of both solutions was at 423 nm. The standard absorbance value of curcumin was higher than turmeric extract. This result was caused by the curcumin content in curcumin standard was far more than the amount of curcumin in curcumin extract.

The peak wavelength of diode laser used in this study was 403 nm, then the absorbance value of curcumin extract was 0.475. The transmittance value of the curcumin

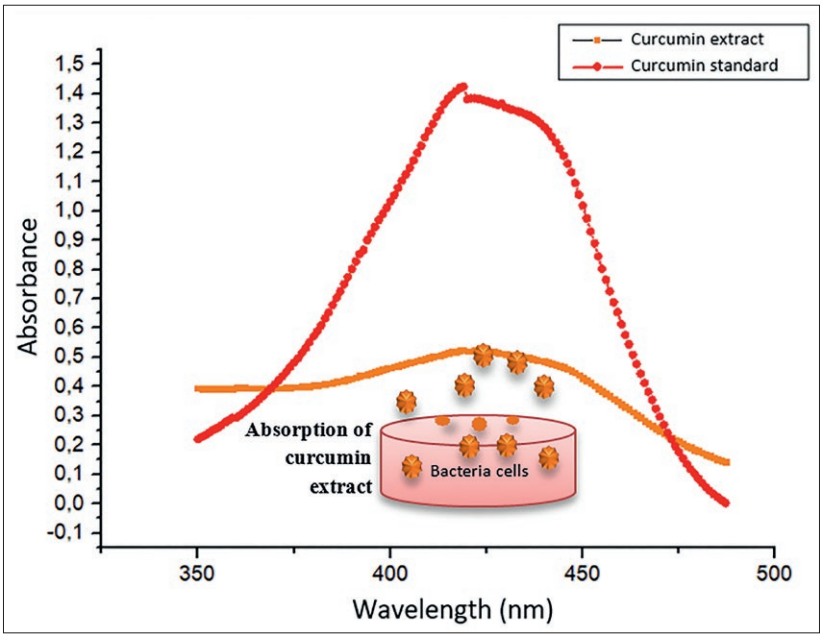

Fig. 1. The absorbance of curcumin extract and curcumin standard
Рис.1. Спектры поглощения экстракта куркумина и стандарта куркумина

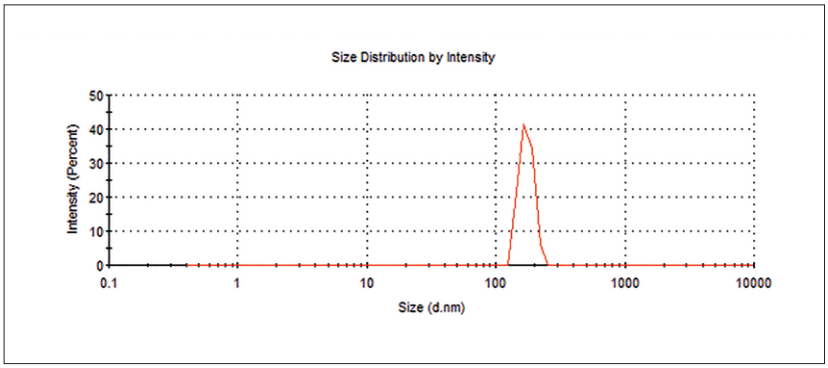

Fig. 2. Nanodoxycycline particle size distribution
Рис.2. Распределение частиц нанодоксициклина по размеру

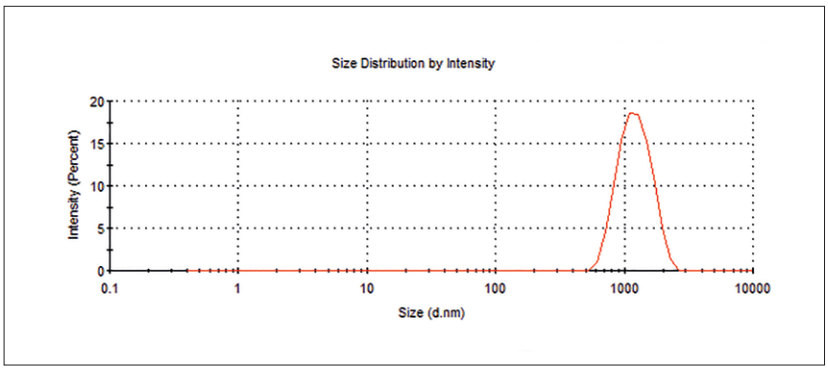

Fig. 3. Doxycycline particle size distribution
Рис.3. Распределение частиц доксициклина по размеру

extract molecule and its absorption percentage can be determined using Lambert-Beer law as follows.

$$A_1 = -\log T_1$$

$$0.475 = -\log T_1$$

$$T_1 = 0.3349$$

So, the absorption of curcumin extract is (1–0.33349) × 100% = 66.51%.

Nanodoxycycline

The 0.1% nanodoxycycline solution was the non-organic exogenous PS used in this study. The goal of reducing doxycycline particles to the nanoscale is that the molecules have a larger surface area to be more easily absorbed by the *A. actinomycetemcomitans*. The value of particle size distribution is at 141.80–220.20 nm, while the doxycycline particle distribution is at 1253.00 nm when tested using Particle Size Analyzer (PSA). The particle size distribu-

Table 1
Analysis of the Nanodoxycycline FTIR Test
Таблица 1
Результаты анализа нанодоксициклина FTIR-тестом

Peak Wavenumber (cm–1)		Functional Group wavenumber values (cm^{-1}) Значения волновых чисел функциональной группы, $см^{-1}$	Functional Group Функциональная группа
Nano doxycycline Нанодоксициклин	Doxycycline [20] Доксициклин		
3525.88	3454	3650-3400	Primary –OH group Основная -OH группа
3336.85	3300	3500-3100	–NH group –NH группа
2931.80	2964	3000-2850	C–H stretching C–H растяжение
1658.78	1672	1680-1630	C–O group C–O группа
1610.56	1618	1680-1600	C–C stretches C–C растяжение
1544.98 and 1454.33	1600 and 1400	1600 and 1475	aromatic C=C bonds ароматические C=C связи
1244.09	1245	1300-1000	C–O bond C–O связь

tion chart of the particle size analyzer test results was shown in Fig. 2 and 3.

The Fourier Transform Infrared (FTIR) test was analyzed with the results shown in Table 1 to prove that the nanodoxycycline functional groups did not experience a significant change compared with the literature. There was a shift in wavenumber but still within the same range of functional groups.

Through PSA and FTIR tests, it was known that doxycyline and nanodoxycycline had differences in terms of functional group and particle size distribution. In the absorbance test using the Genesys 30 Spectrophotometer, the difference was shown by both the absorbance wavelength and the absorbance value. Doxycycline has the highest absorbance peak, which is 2.702, at 375 nm, while the absorbance peak of nanodoxycycline occurs at a wavelength of 377 nm, which is 3.000. The graph of the absorbance value test results is shown in Fig. 4.

When molecular doxycycline is exposed to laser diode with a peak wavelength of 403 nm, the absorbance value of doxycycline is 0.808. With Lambert-Beer law, we can calculate the transmittance value and the percentage of absorption of a molecular of doxycycline.

$$A_2 = -\log T_2$$

$$0,808 = -\log T_2$$

$$T_2 = 0,1556$$

So, the% absorption of nanodoxycycline is

$$(1-0,1556)\times 100\% = 84,44\%$$

Laser Diode Characterization

The laser diode used in this study has a Gaussian output beam. The relatively small diameter of the laser beam, which was (0.20 ± 0.01) cm^2, allows for a high energy density with minimum exposure time. It is necessary to characterize the laser diode beam temperature using a Digital Constant Multimeter 89 to avoid excessive heating. The result data shows that the beam temperature of the laser output was 32.04 ± 0.02 °C, so it can be ascertained that a decrease in the level of biofilm OD is caused by PDT, not due to the thermal reactions. The characterization of the diode laser was shown in Table 2.

Fig. 5 showed the relationship between the wavelength of the Laser Diode and its output power with the Gaussian approach. It is known that the peak wavelength of the Laser Diode was 403.00 ± 0.24 nm, with a power of 27.65 ± 0.01 mW. The output beam intensity was 138.25 ± 0.01 mW/cm^2 for diameter of the beam of 0.20 ± 0.01 $cm^{2.}$ Photochemical reactions in PDT occurred when exposure times > 1 s and power density were in the mW [31]. Therefore, five variations of the exposure time of biofilms were carried out, as summarized in Table 2.

The curcumin and doxycycline absorption spectrum were depicted at 380–780 nm based on the previous studies [11, 15, 17, 23]. When the doxycycline was in the nanoscale size, the absorption spectrum was

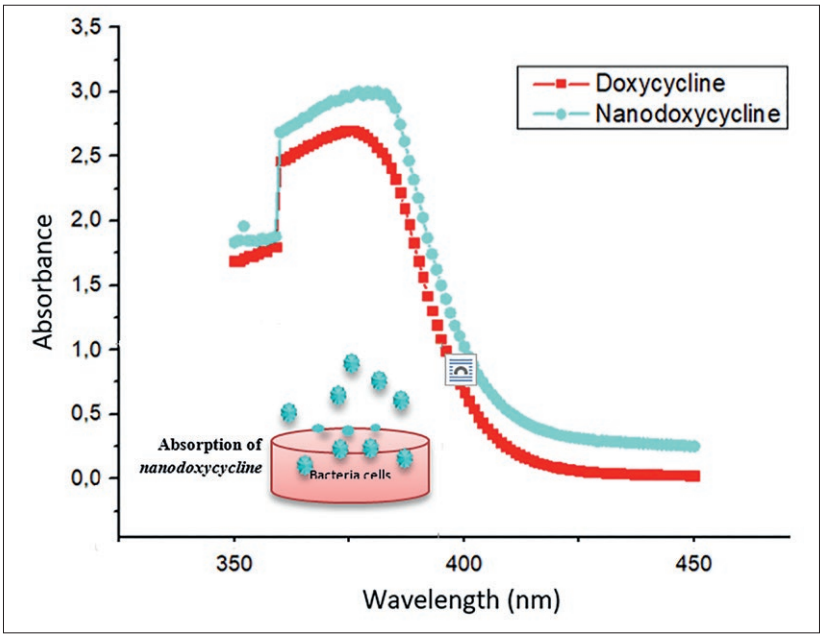

Fig. 4. Absorbance spectrum of doxycycline and nanodoxycycline
Рис.4. Спектры поглощения доксициклина и нанодоксициклина

Table 2
The characterization of laser
Таблица 2
Характеристика лазера

Wavelength (nm) Длина волны, нм	Beam Intensity (mW/cm²) Интенсивность излучения, мВт/см²	Spot Area (cm²) Размер пятна, см²	Time Exposure (s) Время экспозиции, с	Energy Density (J/cm²) Плотность энергии, Дж/см²
			30.000 ± 0.005	4,15
			60.000 ± 0.005	8,29
403,000 ± 0,007	138,25 ± 0,01	0,2±0,01	90.000 ± 0.005	12,44
			120.000 ± 0.005	16,59
			150.000 ± 0.005	20,73

shifted from 375.00 ± 0.05 nm to 377.00 ± 0.05 nm. The level of reactive oxygen formation was affected by this shifting; hence the biofilm reduction escalates too. The wavelength of the laser used in this study was 403.00 ± 0.05 nm. The percentage of photons absorbed by the curcumin and doxycycline was 67% and 84%, respectively.

The wavelength spectrum laser diode corresponds to the absorption spectrum of the exogenous PS used, namely curcumin and nanodoxycycline extracts.

Treatment Results

The results of data analysis showed that diode laser irradiation treatment with endogenous porphyrin, diode laser with curcumin, and diode laser with doxycyclin produced significantly different biofilm reductions ($p < 0.05$). Treatment with diode laser irradiation at various energy densities of 4.15, 8.28, 12.44, 16.59, and 20.73 J/cm² showed no significant difference ($p > 0.05$) in reducing bacterial biofilm. The treatment with diode laser and curcumin, diode laser with nanodoxycyclin, showed a significant difference ($p < 0.05$). Diode laser irradiation of 20.73 J/cm² with an irradiation time of 150 seconds resulted in the greatest reduction of biofilm of 14.94%, diode laser irradiation+Curcumin – 47.82%, and diode laser irradiation+nanodoxycyclin – 53.76%. The results of *A. actinomycetemcomitans* biofilm reduction is shown in Fig. 6.

The photoinactivation mechanism occurs when a biological molecule is exposed to light. A process called photophysical reaction occurs when the energy of photons is absorbed by photosensitizer molecules. The match of the wavelength spectrum between the laser diode and the exogenous PS results in a photophysical process [32]. The diode laser produces energy absorbed by PS molecules so that PS molecules get additional energy to be excited

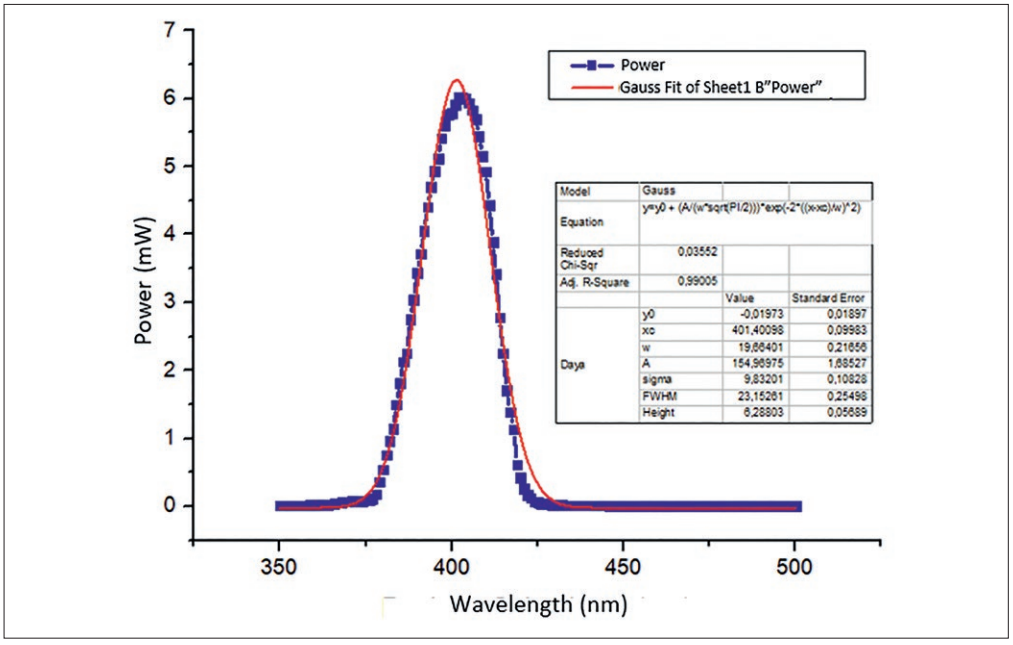

Fig. 5. Blue laser diode characterization
Рис.5. Характеристика диодного лазера

to a higher energy level. This excitation state is unstable so that the PS molecule will return to its ground state, one of which is through photochemical reactions with other molecules in the form of energy transfer or electron transfer [11]. The product of the photochemical reaction is the oxygen radicals (ROS). ROS is reactive and can damage the biofilm cell membrane, thereby disrupting the metabolic activity of cells [33].

In exogenous PS utilization, a type I photochemical reaction occurs when an excited PS molecule initiates a reaction with the substrate to induce photolytic deamination in the exogenous PS fourth carbon ring system. The excited photosensitizer transfers electrons to oxygen to produce superoxide anion (O^{2-}) and forms ROS, which consists of hydroxyl radicals (*OH) and hydrogen peroxide (H_2O_2). Superoxide ionization (O^{2-}) will produce hydrogen peroxide (H_2O_2) and cause a reaction through an oxidation reaction to produce free radicals, causing bio-molecular damage. In a type II reaction, when PS is in triplet state, energy is directly transferred to the oxygen molecule to produce singlet oxygen. Excited singlet oxygen can trigger oxidative peroxidation reactions that damage biological molecules. The last reaction is a photobiological reaction when superoxide is formed in the cell (intracellular) and the peripheral area (extracellular). Hydroxyl radical reactivity causes oxidative damage. Nano-sized doxycycline can diffuse through the pores where the nutrients are transported to all parts of the biofilm region, causing wider biological damage [11].

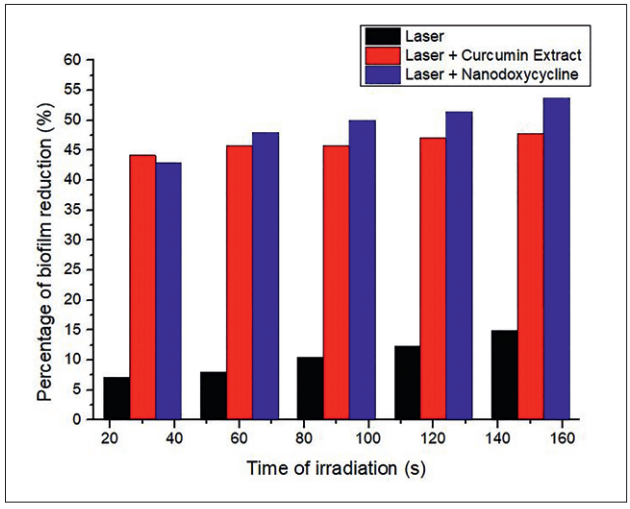

Fig. 6. Histogram Percentage of Biofilm Reduction of *A.actinomycetemcomitans*
Рис.6. Доля уменьшения биопленки *A.actinomycetemcomitans*

Oxidative damage can occur in three different locations based on the photosensitizer, namely the biofilm matrix, cell membrane, and intracellular parts. Biofilm matrix components such as lipids, proteins, and DNA are oxidized by ROS. ROS can interact with the biofilm matrix, which affects the cohesiveness and stability of EPS. It also reacts to lipids on the outside of the cell membrane and changes their morphological structure. It leads to increased photosensitizer intake and stimulation of leakage from cellular metabolites. Damage that occurs in the cell membrane causes inactivation of the membrane transport system. Damage can also occur to intracellular parts such as the nucleus and mitochondria. All of these defects change the phenotype and reduce the biofilm

[11]. The results of the research data analysis showed that the higher the energy density used, the higher the biofilm reduction. The highest decrease occurred in the use of an energy density of 20.73 J/cm^2 for each treatment.

Porphyrins as endogenous PS can reduce biofilms when PDT is used using a diode laser. The highest reduction in biofilm occurred at an energy density of 20.73 J/cm^2 of 14.94%. Porphyrin has an absorption wavelength at 400–450 nm [13], so the diode laser'wavelength used in this treatment is suitable. The relatively low biofilm reduction was due to the limited laser diode penetration of the *A. actinomycetemcomitans* biofilm layer [34]. Due to the limited light penetration, the biofilm layer at the bottom of the plate does not receive the laser diode'energy. Therefore, the ROS only reduced *A. actinomycetemcomitans* biofilm by an average of 14.94%.

The addition of exogenous PS is more effective in reducing biofilms than using only endogenous PS. As an exogenous PS, curcumin extract is relatively sufficient to reduce biofilms. One of the active components of this photosensitizer is curcumin, capable of producing ROS when exposed to light with a wavelength of more than 400 nm [35]. The average reduction in biofilm due to PDT with PS curcumin extract was 46.12%. PDT using curcumin extract was shown to be able to reduce biofilms greater than endogenous PS. However, with a greater energy density, exogenous PS nanodoxycycline was better able to reduce biofilms higher than curcumin PS.

Tetracyclines are well-established antibiotics but exhibit phototoxicity as a side effect. Anti-microbial photodynamic inactivation uses tetracyclines combined with harmless light to destroy microbial cells by reactive oxygen species. Tetracyclines (demeclocycline and doxycycline) can act as light-activating antibiotics by binding to bacterial cells and killing them only after illumination. The remaining tetracyclines can prevent bacterial regrowth after illumination has stopped. Bacteria are killed by photoactivation of tetracyclines without oxygen. Because topical tetracyclines are already used clinically, activation of blue light can increase the bactericidal effect [36].

The ability of biofilms to form extracellular matrices makes the penetration of external particles more difficult [37]. In this study, nano doxycycline particles had a wider surface area, allowing them to be more easily absorbed by *A. actinomycetemcomitans* biofilms. Although biofilms have high resistance to tetracycline antibiotics [38], through the PDT process, it has been shown that the use of antibiotics as PS increases biofilm reduction. The average reduction in biofilm *A. actinomycetemcomitans* with diode laser irradiation of 20.73 J/cm^2 and PS doxycycline was 53.76%.

In addition, the age of the biofilm affects the ability of PDT to reduce biofilms. Previous research has shown that the older the biofilm, the lower the bacterial and biofilm reduction is [39]. This behavior may be due to the limited light penetration of the biofilm. Thus, antibiotics as PS in PDT can be a therapeutic solution due to bacterial infection.

Conclusion

The results of data analysis showed that diode laser irradiation treatment with endogenous porphyrin, diode laser with Curcumin, and diode laser with nanodoxycyclin produced significantly different biofilm reductions. Treatment with diode laser irradiation at various energy densities of 4.15, 8.28, 12.44, 16.59, and 20.73 J/cm^2 showed no significant difference in reducing bacterial biofilm. The treatment with diode and curcumin, and diode laser irradiation with nanodoxycyclin showed a significant difference. Diode laser irradiation of 20.73 J/cm^2 with an irradiation time of 150 seconds resulted in the most significant reduction of biofilm of 14.94%, diode laser irradiation+Curcumin – 47.82%, and diode laser irradiation+nanodoxycyclin – 53.76%. Therefore, PDT using a blue diode laser combined with exogenous PS extract of curcumin and nanodoxycycline is more effective to reduce bacterial biofilms *A. actinomycetemcomitans*.

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A STUDY OF THERAPEUTIC EFFECTS OF 670 NM IRRADIATION IN DIFFERENT TYPES OF DIABETIC MACULAR EDEMA

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Abstract

The purpose of this study was to investigate the therapeutic effects of 670 nm irradiation in patients with diabetic macular edema. In several studies, positive effects of red/near-infrared irradiation showed in a range of ocular diseases such as macular degeneration, macular edema, and retinitis pigmentosa. This study was conducted on forty five eyes of 26 diabetic patients with macular edema between the ages of 51 and 80. Measurement of visual acuity and slit lamp examination, funduscopy, and optical coherence tomography were performed in all subjects. None of the patients had proliferative retinopathy. We used a portable LED device (Warp 10, Quantum Devices) for treatment. Patients held this device at a distance of 3 cm from their eyes for 240 seconds for three months. Full ophthalmic examinations were repeated 1, 2, and 3 months after treatment. After 3 months, the mean visual acuity improved from 0.44 ± 0.38 log MAR to 0.27 ± 0.24 log MAR and vision increased by 1.52 ± 1.16 lines post treatment ($p<0.001$). The mean central macula thickness decreased from 381.49 ± 144.40 μm to 359.72 ± 128.84 μm ($p=0.050$). In patients with mild and moderate nonproliferative diabetic retinopathy, the mean central retinal thickness decreased 52.06 ± 67.78 μm and 39.27 ± 44.69 μm, respectively, but patients with severe type showed an increase of 34.93 ± 65.65 μm in the mean central retinal thickness ($p<0.001$). Also, the severity of macular edema had no effect on final outcomes ($p>0.05$). Photobiomodulation can positively affect diabetic macular edema, especially in patients with mild to moderate diabetic retinopathy.

Keywords: diabetic macular edema, photobiomodulation, diabetic retinopathy.

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ИЗУЧЕНИЕ ТЕРАПЕВТИЧЕСКИХ ЭФФЕКТОВ ИЗЛУЧЕНИЯ С ДЛИНОЙ ВОЛНЫ 670 НМ ПРИ РАЗЛИЧНЫХ ТИПАХ ДИАБЕТИЧЕСКОГО МАКУЛЯРНОГО ОТЕКА

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Резюме

Целью данной исследовательской работы являлось изучение терапевтических эффектов излучения с длиной волны 670 нм у пациентов с диабетическим макулярным отеком. Ряд предыдущих исследований свидетельствует о положительном эффекте красного / инфракрасного излучения при некоторых заболеваниях глаз, таких как макулодистрофия (дегенерация желтого пятна), макулярный отек и пигментный ретинит. Наше исследование было проведено на 45 глазах у 26 больных сахарным диабетом в возрасте от 51 до 80 лет с макулярным отеком. Всем пациентам были проведены определение остроты зрения, осмотр глаз щелевой лампой, фундоскопия и оптическая когерентная томография. Ни у одного из пациентов не было пролиферативной ретинопатии. Для лечения нами был применен портативный светодиодный прибор (Warp 10, Quantum devices). Пациенты держали светодиод на расстоянии 3 см от глаза в течение 240 сек в течение 3 мес. Все офтальмологические исследования были повторены через 1, 2 и 3 мес после проведения лечебной процедуры. Через 3 мес средняя острота зрения улучшилась с показателем логарифма минимального угла разрешения 0,44 ± 0,38 до 0,27 ± 0,24, что показало увеличение показателя остроты зрения на 1,52 ± 1,16 после лечения ($p<0,001$). Средняя центральная толщина сетчатки в области макулы уменьшилась с 381,49 ± 144,40 мкм до 359,72 ± 128,84 мкм ($p=0,050$). У пациентов с легкой и умеренной непролиферативной диабетической ретинопатией средняя толщина сетчатки уменьшилась до 52,06 ± 67,78 и 52,06 ± 67,78 мкм, соответственно, а у пациентов с тяжелой ретинопатией наблюдалось увеличение на 34,93 ± 65,65 мкм ($p<0,001$). Помимо

того, степень макулярного отека не повлияла на окончательный результат лечения (p>0,05). Фотобиомодуляция была эффективной при диабетическом макулярном отеке, в частности, у пациентов с легкой и умеренной диабетической ретинопатией.

Ключевые слова: диабетический макулярный отек, фотобиомодуляция, диабетическая ретинопатия.

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Introduction

According to International Diabetes Federation (IDF) reports, the global prevalence of diabetes among adults over 18 was 8.4% in 2017. The number of patients is expected to increase to 693 million by 2045 [1]. Visual impairment due to diabetes is a major global health problem in the world. Diabetic macular edema (DME) and diabetic retinopathy are the main causes of visual impairment in these patients affecting their activities and lives [2–3]. Chronic hyperglycemia causes the generation of advanced glycation endproducts (AGEs), activation of protein kinase C, upregulation of vascular endothelial growth factor (VEGF), vascular endothelial dysfunction, and increased vascular permeability and chronic inflammation [4–5]. Treatment modalities include laser photocoagulation and anti-vascular endothelial growth factor (VEGF) drugs with aggressive control of glycemia. Although these methods have been effective against macular edema, they have disadvantages such as decreased vision in some patients, a need for repeated injections, and high costs [6–8].

Several studies demonstrated the therapeutic effects of red to near-infrared light (NIR) (630–1000 nm) by using low-level lasers or light-emitting diode (LED) arrays. Light photons can penetrate into living tissues, and the absorbed energy creates photochemical changes within cellular structures that define photobiomodulation (PBM) therapy. Photobiomodulation therapy affects endogenous chromophores in the body and improves the biological functions of cells without heating or damage [9–12]. In the injured optic nerve, this approach has shown the potential to reduce inflammation and alleviate degeneration. Following photo-irradiation of nervous cells, cytochrome oxidase production is increased, and the activity of the cytochrome oxidase inhibitors is reduced [13]. In another study, NIR irradiation of the optic nerve following an injury in a transcranial manner reduced oxidative stress. Reduced oxidative stress by NIR light improved function in the CNS post traumatic injury in vivo [14]. Photobiomodulation has shown positive effects in the treatment of strokes and myocardial infarction, and stem cell proliferation [15]. The first study of photobiomodulation efficacy in the treatment of dry age-related macular degeneration (AMD) was conducted by Merry et al. They demonstrated improvements of functional and anatomical outcomes in their subjects with PBM therapy [16]. PBM effects on diabetic macular edema was studied by Tang in a pilot study. Daily photobiomodulation caused a significant reduction in focal retinal thickening and improved vision in treated eyes. He reported PBM as an effective and non-invasive method to treat diabetic macular edema lesions. [17]. In previous studies, PBM was reported as a safe method without side effects [9–10, 17].

According to this evidence, photobiomodulation might have positive effects in diabetic macular edema. Therefore, the present study is designed to evaluate whether 670 nm irradiation has therapeutic effect in diabetic macular edema, focusing on different types of edema and several stages of nonproliferative diabetic retinopathy.

Materials and Methods

This study was conducted on 26 diabetic patients in Abhar, Iran, in 2019. Our study followed the tenets of the Declaration of Helsinki and was approved by the Human Ethics Committee of Shahid Beheshti University of Medical Sciences (IR. SBMU. RETECH. REC. 1398.558). After approval by the human ethics committee of the University, diabetic patients were recruited from an eye clinic. All patients had diabetic macular edema and associated decreased visual acuity. Only patients who did not wish to receive the standard treatment or had not responded to current modalities participated in this study. In our patients, visual acuity measurement with ETDRS chart, slit lamp examination, funduscopy, and optical coherence tomography were performed. According to the International Clinical Diabetic Macular Edema Disease Severity Scale [18], diagnosis of diabetic macular edema was approved on the basis of clinical findings and optical coherence tomography (OCT SD) data. Exclusion criteria were a history of systemic or topical anti-inflammatory drugs usage, intravitreal injections of steroids and anti-VEGF, focal laser therapy within 1 year, and evidence of proliferative diabetic retinopathy. The treatment method was described to all patients, and the consent form was received from them. Photobiomodulation therapy was applied using a portable LED device Warp 10 (Quantum Devices).

This device emits red light at a wavelength of 670 nm with 25 J/cm^2 energy in 3 cm distance to an eye. The duration of treatment was three months, and it was carried out at home. Our patients would keep this device at a distance of 3 cm from their eyes for 240s three times a week in the first month. In the following month, they performed photobiomodulation two times a week, and in the last month, they continued it weekly. After three months, all examinations were repeated, and changes in ocular findings were considered as the final outcomes.

Statistical analysis of results was performed by SPSS software version 18. After the assessment of normality of data distribution with the Shapiro-Wilk test, we used Student's, Wilcoxon, and Kruskal-Wallis tests for statistical analysis of results.

Results

Twenty six patients (11 male and 15 female) with a mean age of 63.44 ± 7.51 (range 51–80) years participated in this study. During this study, routine treatment of diabetes, including drugs and diet, were not changed in all patients. Mean FBS in the first and final examinations were 200.11 ± 54.68 and 193.35 ± 61.25, respectively. The mean spherical equivalent was 0.68 ± 0.89 diopter (D) with range –2.0 to +3.25 D. Photobiomodulation was performed in both eyes of 19 subjects and did in one eye of 7 patients. Eighteen eyes had mild nonproliferative diabetic retinopathy (NPDR), 13 eyes had moderate nonproliferative diabetic retinopathy, and 14 eyes were in a stage of severe NPDR. In terms of severity of disease, DME in 8 eyes was mild, in 15 eyes was moderate, and in 22 eyes was severe. According to morphology of edema, 8 patients had simple edema, and 26 patients had cystoid type, and 11 of them had neuroretinal detachment.

Initially, the mean visual acuity of patients was 0.44 ± 0.38 log MAR that improved to 0.29 ± 0.25 log MAR, 0.26 ± 0.28 log MAR, and 0.27 ± 0.24 log MAR after one, two, and three months, respectively ($p < 0.001$). The mean visual acuity in patients increased 1.52 ± 1.16 lines after 3 months. While visual acuity improved in 67% of subjects (between 1–2 lines in 19 eyes (42%) and more than 2 lines in 11 eyes (25%)), no positive effects were observed in 33% of eyes (no change in visual acuity in 9 eyes (20%), and decreased visual acuity in 6 eyes (13%)). The mean central retinal thickness was 381.49 ± 144.40 µm primarily and decreased to 359.72 ± 128.84 µm after 3 months (p=0.050) (figures 1–3). At baseline, the mean retinal thickness in 3 mm central circle was 404.16± 91.15 µm that decreased to 390.24 ± 97.87 µm after treatment (p=0.004) and the mean retinal thickness in 6 mm central circle was 367.54 ± 76.37 µm primarily that decreased to 356.31± 83.03 µm finally (p=0.002). The mean retinal thickness decreased 20.47± 72.20 µm (range: from 275 µm decrease to 121 µm increase in thickness), 14.01 ± 40.13 µm (range: from 130 µm decrease to 118 µm increase in thickness), and 11.21 ± 39.22 µm (range: from 155 µm decrease to 136 µm increase in thickness) in the central, 3 mm circle, and 6 mm circle respectively (Table 1).

According to changes of the retinal thickness, patients were divided in three groups: stable, decrease, and increase. Nine eyes (20%) had no change in the central retinal thickness (±10.00 µm changes in the retinal thickness). Twenty five eyes (56%) showed reduction of the central retinal thickness (from 10 to 50 µm decrease of the mean retinal thickness in 14 eyes (31%) and from 50 to 275 µm decrease of the mean retinal thickness in 11 eyes (25%)). Also, the central retinal thickness increased in 11 eyes (24%) (from 10 to 50 µm increase of mean retinal thickness in 6 eyes (13%) and from 50 to 121 µm increase of mean retinal thickness in 5 eyes (11%)).

The severity of macular edema had no effect on final outcomes (p>0.05). Central retinal thickness decreased 12.25 ± 45.01 µm, 17.15 ± 33.30 µm, and 25.41± 95.17 µm in mild, moderate, and severe macular edema respectively (Table 2). The morphology of macular edema had significant effect on central retinal thickness (p=0.01). The mean central retinal thickness decreased 19.50 ± 26.36 µm and 41.23 ± 70.84 µm in simple and cystoid macular edema, respectively, but it increased 28.09 ± 72.71 µm in neuroretinal detachment macular edema (Table 3).

Furthermore, the rate of central retinal thickness changes depended on the severity of diabetic retinopathy. In patients with mild and moderate NPDR, the mean central retinal thickness decreased 52.06 ± 67.78 µm and 39.27 ± 44.69 µm, respectively, but patients with severe NPDR showed the rate of 34.93 ± 65.65 µm increase in the mean central retinal thickness (p<0.001). Fourteen eyes (78%) with mild NPDR and 8 eyes (61%) with moderate NPDR showed a reduction of macular thickness between 10 to 273 µm. Of the total 14 eyes with severe NPDR, 3 eyes (21%) had 10 to 135 µm decrease of central retinal thickness, and 10 eyes (70%) had 10 to 121 µm increase of central retinal thickness. Eight patients (15 eyes) had a history of previous anti-VEGF injection, and their results showed no difference from other patients (p>0.05). Finally, our subjects showed no adverse events such as blurred vision, inflammation, or increased intraocular pressure after photobiomodulation.

Discussion

Evidence is growing that photobiomodulation has beneficial effects in a variety of diseases, including wound healing, rheumatoid arthritis, cerebral degeneration, Alzheimer's disease, and retinal degeneration [19,20]. In several studies, therapeutic effects of PBM were investigated in the field of ocular diseases such as age-related macular degeneration (AMD), diabetic macular edema, and retinitis pigmentosa [17, 21–23]. Albarracin et al. showed protective effect of NIR light in

Table 1
Results of photobiomodulation at baseline and during the study
Таблица 1
Результаты фотобиомодуляции до и во время исследования

Results Результаты	Baseline Исходный уровень	3th month 3-й месяц	p
Best Corrected Visual Acuity Наилучшая скорректированная острота зрения	0.44 ± 0.38 Log MAR	0.27 ± 0.24 Log MAR	<0.001
Mean central macular thickness Толщина макулы в центре	381.49 ± 144.40 μm	359.72 ± 128.84 μm	0.050
Mean minimum central macular thickness Минимальная толщина макулы в центре	332.60 ± 117.37 μm	309.17 ± 113.09 μm	0.005
Mean maximum central macular thickness Максимальная толщина макулы в центре	461.51 ± 130.01 μm	438.26 ± 125.92 μm	0.087
Mean 3 mm central macular thickness Толщина макулы в 3 мм от центра	404.16 ± 91.15 μm	390.24 ± 97.87 μm	0.004
Mean 6 mm central macular thickness Толщина макулы в 6 мм от центра	367.54 ± 76.37 μm	356.32 ± 83.04 μm	0.002

Table 2
Results of photobiomodulation according to Macular Edema Severity
Таблица 2
Результаты фотобиомодуляции в соответствии со степенью тяжести отека желтого пятна

Macular Edema Макулярный отек	Visual acuity improvement (line) Улучшение остроты зрения	Retinal thickness changes in the center (μm) Изменения толщины сетчатки в центре (мкм)	Retinal thickness changes 3 mm from the center (μm) Изменения толщины сетчатки в 3 мм от центра (мкм)	Retinal thickness changes 3 mm from the center (μm) Изменения толщины сетчатки в 6 мм от центра (мкм)
Mild Edema **Слабый отек** **n=8 eyes**	2.07 ± 0.98	−12.25 ± 45.01	−5.38 ± 5.71	−12.25 ± 10.21
Moderate Edema **Умеренный отек** **n=15 eyes**	1.50 ± 104	−17.15 ± 33.30	−12.77 ± 29.44	−15.54 ± 22.80
Severe Edema **Сильный отек** **n=22 eyes**	1.32 ± 1.27	−25.41 ± 95.17	−18.15 ± 52.73	−8.00 ± 53.36
p	0.339	0.952	0.616	0.503

Table 3
Results of photobiomodulation according to macular edema morphology
Таблица 3
Результаты фотобиомодуляции в соответствии с морфологией отека желтого пятна

Macular Edema Макулярный отек	Visual acuity improvement (line) Улучшение остроты зрения	Retinal thickness changes in the center (μm) Изменения толщины сетчатки в центре (мкм)	Retinal thickness changes 3 mm from the center (μm) Изменения толщины сетчатки в 3 мм от центра (мкм)	Retinal thickness changes 3 mm from the center (μm) Изменения толщины сетчатки в 6 мм от центра (мкм)
Simple Edema **Простой отек** **n=8 eyes**	1.75 ± 1.10	−19.50 ± 26.36	−7.50 ± 4.81	−12.17 ± 7.63
Cystoid Edema **Цистоидный отек** **n=26 eyes**	1.88 ± 0.89	−41.23 ± 70.84	−24.86 ± 37.41	−24.71 ± 35.88
Neuroretinal detachment **Нейроретинальная отслойка** **n=11 eyes**	0.49 ± 1.24	+28.09 ± 72.71	+6.36 ± 49.46	18.73 ± 41.69
p	0.010	0.014	0.028	0.001

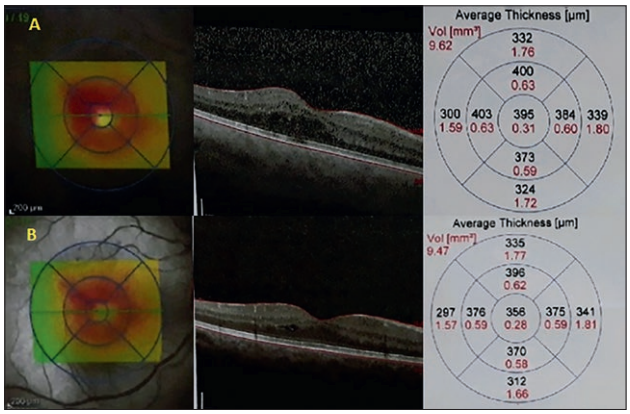

Fig. 1. A: Pre Photobiomodulation OCT of a patient with BCVA: 0.22 Log MAR. B: Post photobiomodulation OCT of same patient with BCVA: 0.05 Log MAR after three months.
Рис. 1. A: Оптическая когерентная томография перед фотобиомодуляцией пациента с наилучшей коррегируемой остротой зрения: логарифм минимального угла разрешения 0,22; B: Оптическая когерентная томография после фотобиомодуляции пациента с наилучшей коррегируемой остротой зрения: через 3 мес логарифм минимального угла разрешения 0,05

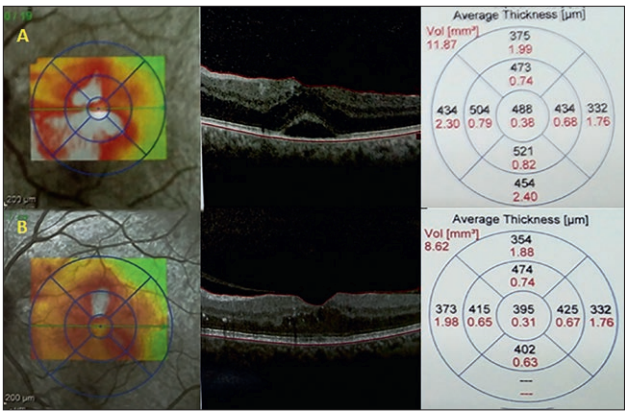

Fig.2. A: Pre photobiomodulation OCT of another patient with BCVA: 0.80 Log MAR. B: Post photobiomodulation OCT of same patient with BCVA: 0.30 Log MAR after 3 months.
Рис. 2. A: Оптическая когерентная томография перед фотобиомодуляцией другого пациента с наилучшей коррегируемой остротой зрения: логарифм минимального угла разрешения 0,80; B: Оптическая когерентная томография после фотобиомодуляции пациента с наилучшей коррегируемой остротой зрения: через 3 мес логарифм минимального угла разрешения 0,30

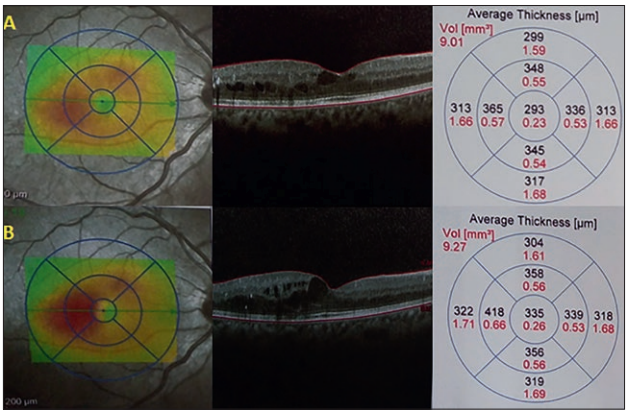

Fig. 3. A: Pre photobiomodulation OCT of another patient with BCVA: 0.20 Log MAR. B: Post photobiomodulation OCT of same patient with BCVA: 0.25 Log MAR after 3 months.
Рис. 3. A: Оптическая когерентная томография перед фотобиомодуляцией пациента с наилучшей коррегируемой остротой зрения: логарифм минимального угла разрешения 0,20; B: Оптическая когерентная томография после фотобиомодуляции пациента с наилучшей коррегируемой остротой зрения: через 3 мес логарифм минимального угла разрешения 0,25

the albino rat retina from light-induced photoreceptor degeneration. This protective effect appears to involve a reduction of cell death and inflammation [21]. Ivandic and Ivandic evaluated therapeutic effects of photobiomodulation in 203 patients with AMD. Visual acuity improved in 97% of the subjects [22]. Merry et al. studied the efficacy of PBM in 42 eyes with dry AMD. PBM resulted in a significant improvement in mean BCVA and contrast sensitivity. Although drusen volume decreased, overall central retinal thickness and retinal volume remained stable. They reported these results were related to anti-inflammatory, anti-oxidative, neuroprotective, and anti-apoptotic properties of PBM [23]. Tang et al., in another study, demonstrated the efficacy of PBM in four eyes of 4 diabetic patients as increased visual acuity and 20% reduction in macular thickness in all treated eyes [17]. In agreement with previous works, our results showed the beneficial effects of photobiomodulation. In 67% of subjects, vision improved, and visual acuity increased more than 1.50 lines after treatment. Improvement of vision occurred for two months and then remained stable for the third month. Positive effects of PBM were observed in all three types of mild, moderate, and severe types of macular edema. In our study, the rate of improvement was related to the morphology of edema. Cystoid form of edema showed a better response to PBM associated with decreased honeycomb spaces. Macular thickening after PBM decreased to approach near normal architecture, and retinal layers were more regularly arranged. Interestingly, the amount of therapeutic effects of PBM on macular edema was dependent on diabetic retinopathy severity. In patients with mild to moderate nonproliferative diabetic retinopathy, macular edema decreased after photobiomodulation. However, in the majority of patients with severe nonproliferative diabetic retinopathy (70%), photobiomodulation could not arrest or regress macular thickening.

Direct and indirect mechanisms of biological effects by PBM are still under investigation. Previous studies demonstrated beneficial effects of PBM on the activity of cytochrome oxidase, activation of light-gated ion channels, stem cell proliferation, and anti-inflammatory actions [24]. Activity and expression of cytochrome oxidase in retinas of diabetic rats was not affected by

PBM. Although, some studies showed increased cytochrome oxidase activity in retinal pigment epithelium [19, 25]. The beneficial effects of PBM on stem cells have been investigated in several studies [19, 24, 26]. Proliferation of mesenchymal stem cells and cellular viability was enhanced by multiple exposures to 630-nm LEDs [26]. In diabetic mice treated with PBM, the number of c-Kit$^+$ cells in the circulation increased, which was related to a significant effect of photobiomodulation on stem cells [19]. However, the lack of accumulation of c-Kit$^+$ cells within the neural retina or retinal vasculature demonstrated no positive effect of PBM on stem cells in the retina in diabetes [19, 24, 27]. Several studies showed that photobiomodulation inhibited the oxidative stress and inflammation development in the diabetic retina, as well as upregulating survival pathways [25, 28]. The pathogenesis of diabetic retinopathy is related to oxidative stress (upregulation of reactive oxygen species (ROS)) and inflammatory changes (increased pro-inflammatory cytokines and nitric oxide) in the retina [29]. Oxidative stress-induced damage of mitochondrial DNA leads to impaired transcription of electron transport chain proteins, which compromises electron transport chain function and further intensifies ROS production [30]. Also, leukocyte adhesion and endothelial cell death cause the structural and functional abnormalities related to diabetic retinopathy [24, 31]. Heo et al. assessed the anti-oxidative effect LED of 660 nm in hippocampal cell line and the activation of cAMP response element. Photobiomodulation therapy inhibited apoptosis of hippocampal cells induced by oxidative stress and increased neurotrophic factor expression [32]. de Oliveira et al. studied oxidative stress markers following low-intensity laser therapy on rats subjected to a high-intensity resistive exercise session. They stated that LLLT prior to resistive exercise reduced the oxidative stress markers and increased the antioxidant capacity [33]. In another study, Saliba et al. assessed the protective effects of far-red light exposure against retinal oxidative stress and inflammation in diabetic mice. PBM improved diabetes-induced changes in superoxide generation, leukostasis, expression of ICAM-1 (intercellular adhesion molecule-1). Also, in assessments, PBM enhanced both inner and outer retinal uptake of manganese, and ion channel function secondary to inhibition of the oxidative stress [34]. Finally, some studies showed an indirect effect of photobiomodulation. Because of the deep penetration of far-red light into tissues, beneficial effects of PBM via systemic mediators in kidney and heart of diabetic animals and skin wounds have been seen [34–36]. This effect of PBM in diabetic macular edema has not yet been investigated, but due to the close proximity of the eyes, there may be an indirect effect of photobiomodulation that needs further evaluation. In addition, our findings indicated that the severity of diabetic retinopathy influenced on therapeutic effects of PBM. In the severe stage of diabetic retinopathy, vascular abnormalities result in retinal ischemia, with a release of proangiogenic factors and enhanced expression of VEGF [37]. It has been recently revealed that half of the patients with severe nonproliferative diabetic retinopathy have small preretinal neovascularization not seen on clinical examination or OCT [38]. It seems to be essential to use anti-VEGF drugs in severe cases.

The limitation of this study is that we had no control group. Moreover, we could not assess vascular changes after PBM using OCTA. We suggest further studies with more patients and long-term follow-up and combined other therapies.

Conclusion

According to our results, PBM can positively affect diabetic macular edema, especially in patients with mild to moderate diabetic retinopathy. Three months of PBM improves the visual function of diabetic patients and reduces macular edema by anti-oxidative stress and anti-inflammatory actions. Also, this method is a non-invasive and inexpensive method administered at home.

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INTRAOPERATIVE PHOTODYNAMIC THERAPY AND HYPERTHERMIC INTRAPERITONEAL CHEMOTHERAPY IN CYTOREDUCTIVE TREATMENT OF PATIENTS WITH DISSEMINATED MUCINOUS CARCINOMA OF APPENDIX

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Abstract

The article presents the experience of surgical treatment of 57 patients with peritoneal pseudomyxoma of appendicular genesis. In 32 (56.1%) patients, the operation was supplemented with intraoperative photodynamic therapy (IOPDT). In the other 25 (43.9%) patients, hyperthermic intraperitoneal chemotherapy (HIPEC) was performed. The analysis according to the value of the peritoneal carcinomatosis index, completeness of cytoreduction, the volume of operations performed, postoperative complications and hospital mortality, as well as long-term treatment results in two groups is presented. It was shown that with significantly worse results in terms of cytoreduction completeness obtained in the IOPDT group compared to the HIPEC group, the 5-year survival rate in the HIPEC group was 86.6%, with IOPDT - 65.2%. At the same time, in the IOPDT group, the rate of postoperative complications was significantly lower (11.1%), and there was no mortality; in the HIPEC group, these indicators were 23.8% and 12.0%, respectively. The results obtained indicate that the IOPDT method is an effective and promising direction in the surgical treatment of peritoneal pseudomyxoma.

Keywords: peritoneal pseudomyxoma, photodynamic therapy, hyperthermic chemotherapy, peritoneal carcinomatosis index, cytoreductive surgery.

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ИНТРАОПЕРАЦИОННАЯ ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ И ГИПЕРТЕРМИЧЕСКАЯ ВНУТРИБРЮШНАЯ ХИМИОТЕРАПИЯ ПРИ ЦИТОРЕДУКТИВНОМ ХИРУРГИЧЕСКОМ ЛЕЧЕНИИ БОЛЬНЫХ ДИССЕМИНИРОВАННОЙ МУЦИНОЗНОЙ КАРЦИНОМОЙ АППЕНДИКСА

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Резюме

В статье представлен опыт хирургического лечения 57 больных псевдомиксомой брюшины аппендикулярного генеза. У 32 (56,1%) пациентов операция дополнена интраоперационной фотодинамической терапией (ИОФДТ), у других 25 (43,9%) больных проведена

гипертермическая внутрибрюшная химиотерапия (ГИВХ). Представлен анализ по величине индекса перитонеального канцероматоза, полноте циторедукции, обьему выполненных операций, послеоперационным осложнениям и госпитальной летальности, отдаленным результатам лечения в двух группах. Показано, что при достоверно худших результатах по показателям полноты циторедукции, полученных в группе ИОФДТ по сравнению с группой ГИВХ, 5-летняя выживаемость в группе с ГИВХ составила 86,6%, с ИОФДТ – 65,2%. При этом в группе ИОФДТ был достоверно ниже показатель послеоперационных осложнений (11,1%) и отсутствовала летальность, в группе ГИВХ эти показатели составили 23,8% и 12,0%, соответственно. Полученные результаты свидетельствуют о том, что метод ИОФДТ является эффективным и перспективным для использования при хирургическом лечении псевдомиксомы брюшины.

Ключевые слова: псевдомиксома брюшины, фотодинамическая терапия, гипертермическая химиотерапия, индекс перитонеального карциноматоза, циторедуктивная операция.

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Introduction

Tumors of the vermix account for less than 1% of all malignant neoplasms. The most common morphological forms of tumors of this localization are mucinous carcinomas (about 50%), intestinal adenocarcinomas (10%), signet ring cell carcinoma and neuroendocrine tumors [1]. The progression of low-grade mucinous neoplasia leads to the development of peritoneal pseudomyxoma, which is currently considered a clinical syndrome, rather than a pathomorphological definition. In pseudomyxoma, mucinous masses of different densities (soft, semihard, hard) accumulate in the abdominal cavity with a different ratio of tumor cells and different degree of invasion into the parietal and visceral sheets of the peritoneum [2]. In the 8th edition of the TNM Classification of Malignant Tumors, in the determination of the stage of mucinous tumors of the appendix, the primary tumor is assessed from T4a to T4b; distant metastases: M1a: peritoneal, mucin only, without tumor cells, and M1b: peritoneal mucinous carcinomatosis [3]. Regional lymph nodes involvement is hardly ever observed in this disease. Cytoreductive surgery in patients with low-grade mucinous tumors allows achieving 5-year survival rates of 50-85%, depending on the TNM level of the process. In cases of non-mucinous adenocarcinomas of the appendix, the indicator does not exceed 10% [4]. In recent years, a number of authors suggest using photodynamic therapy, which produces a multivariate antitumor response, to increase the effectiveness of cytoreductive surgery [5–8].

We present our experience of surgical treatment of peritoneum pseudomyxoma with the use of intraoperative photodynamic therapy (IOFDT) and hyperthermic intraperitoneal chemotherapy (HIPEC).

Materials and methods

Surgical treatment was performed in 57 patients with peritoneum pseudomyxoma of appendiceal origin. The criteria for inclusion in the study were verified mucinous neoplasia of the appendix after appendectomy or the morphological pattern of pseudomyxoma obtained by biopsy, as well as the absence of other types of abdominal and pelvic tumor pathology. The majority of patients were female (40 people). The age of patients ranged from 25 to 79 years, the average age being 52.5±11.7 years, the majority of patients belonged to the capable to work and socially active population. In 32 (56.1%) patients, cytoreductive surgery was supplemented with IOFDT, in 25 (43.9%), with HIPEC. There were no significant differences between the study groups in terms of gender and age. Almost a third (29.2%) of patients had previously undergone abdominal surgery, including for pseudomyxoma. The prevalence of the process was estimated by the value of the peritoneal carcinomatosis index (PCI). We used the methods developed at P. A. Hertsen Moscow Oncology Research Center for determining CT PCI and ultrasound PCI, on the basis of which the involvement of each of the 13 sectors of the abdominal cavity was evaluated, as well as the PCI score, the maximum value of which was 39. At low PCI≤5, diagnostic fluorescence laparoscopy was performed . The diagnostic task was not only to determine the PCI value, but also to plan the surgery taking into account the degree of damage to the visceral peritoneal layer, the involvement of elements of the hepatoduodenal ligament and lesser peritoneal sac, as well as the expected scope of cytoreduction.

The specifics of the surgical stage

Surgical intervention began with dissection of the skin, subcutaneous tissue and the aponeurosis along

the white line, without opening the abdominal cavity, if possible. With an electric knife, the peritoneum was separated from the transverse fascia and the posterior layer of the aponeurosis of the rectus muscles as laterally and dorsally as possible, up to the lumbar muscles. Defects of the peritoneal lamina were sutured to avoid the leakage of fluid or mucinous masses. If the changes in the parietal peritoneum were not pronounced, it was mobilized together with the connective tissue structures of the anterior abdominal wall, leaving aponeurotic tape about 1 cm wide at the edges of the incision for subsequent suturing. The upper limit of the mobilization of the parietal peritoneum was the diaphragm, the lower being the bladder and the Douglas pouch. After that, laparotomy was performed. After removal of mucin of various fractions and aspiration of ascitic fluid, the abnormal large omentum underwent subtotal resection or extirpation. Then the colon and the rectosigmoid colon were mobilized in the same way as in subtotal colectomy. In pseudomyxoma, either right-sided hemicolectomy or appendectomy was performed, depending on the degree of changes in the appendix and the dome of the cecum, as well as the scope of involvement. The parietal peritoneum was removed acutely in three parts: the peritoneum of the right lateral canal and the right half of the diaphragm, the left lateral canal with the remaining part of the peritoneum of the diaphragm, and the pelvic part with the visceral layer, starting from the mesenteric root. All tumor implants were removed from the peritoneal omental sac, the Douglas pouch, and the liver capsule, and, when necessary, the abdominal and pelvic organs were resected or removed.

The basis of cytoreductive surgery for carcinomatosis is the maximum possible removal of the parietal layer of the peritoneum, i. e., subtotal parietal peritonectomy, the lesion of the visceral layer being the main obstacle to optimal cytoreduction. The organs that are most frequently extirpated include the uterus with appendages, the spleen, the right half of the colon, and the appendix. Given the specifics of the tumor cells spread, resection of the right dome of the diaphragm and the bladder was necessary in more than half of the cases. The key question in the case of a preserved appendix is the choice of the scope of intervention for its tumor, i. e., right-sided hemicolectomy or appendectomy. It is necessary to take into account the PCI value and the tumor involvement of the caecum.

The intraoperative photodynamic therapy method

IOFDT was performed according to the method developed at the P. Herzen MORC. Two drugs were used as photosensitizers: photohem and photosense. The first was administered intravenously 48 hours before surgery at a dose of 2.5 mg per kg of body weight, the second was 2-8 hours before the start of the radiation session at a dose of 0.2 mg per kg of body weight. A strict light regime was observed by the patients for 4 or 8 weeks, for photohem and photosens, respectively. During the IOFDT session, the abdominal cavity walls, the areas of the parietal peritoneum remaining after surgery, and the organs that were affected but not resected (the stomach, the bladder, the spleen), as well as the edges of the laparotomic wound, were exposed to radiation. To perform the manipulation, a laser installation LFT-630/675-01-Biospec (OOO "Biospec", Russia) was used, with a wavelength of 630 nm (for photohem) and 672 nm (for photosense), a special transducer with an outer diameter of 10 mm, through which a cylindrical diffuser with a diameter of 1.5 mm was passed. At a distance of 2.5 cm from the distal end of the transducer, a special opening is cut on $^1/_2$ of the circumference. The use of this design made it possible to provide the desired direction of the light flow. The colon and the loops of the small intestine were protected by a special light-tight cloth. Sequentially, with the overlap of the light fields, all accessible abdominal areas were treated, on average, about 20 positions with 2.5 minutes exposure for each. The power density of the light energy ranged from 6 to 10 J/cm^2. The "blind" part of the transducer was constantly turned to the loops of the small intestine in order to avoid their specific damage. However, taking into account the time of the resection stage, the visceral peritoneum also received a therapeutic dose of laser radiation.

Hyperthermic intraoperative intraperitoneal chemotherapy method

HIPEC was performed after the completion of the surgical stage, with the use of Coliseum (open) technique. The edges of the wound were attached with ligatures on a circular wound dilator, and more recently on a multi-functional THOMPSON wound retractor. We used the SunCHIP device (GamidaTech, France). As a cytostatic agent, cisplatin was used at a dose of 100 mg/m^2 per 400 ml of saline solution. The operating temperature at the inlet was 43-44°C, at the outlet, 42-43°C. The procedure with the use of the chemical product lasted 60 minutes. During the treatment, the temperature regime in various parts of the abdominal cavity and the patency of the drainage tubes were carefully monitored. Given the cytoreductive nature of the surgery, a large number of small fragments of adipose tissue remain in the abdominal cavity, despite its preliminary sanitation, which disrupts the drainage function of the system and thereby causes local hyperthermia in certain areas of the abdominal cavity. Body temperature was monitored with esophageal and skin sensors, preventing it from rising above 39.5°C.

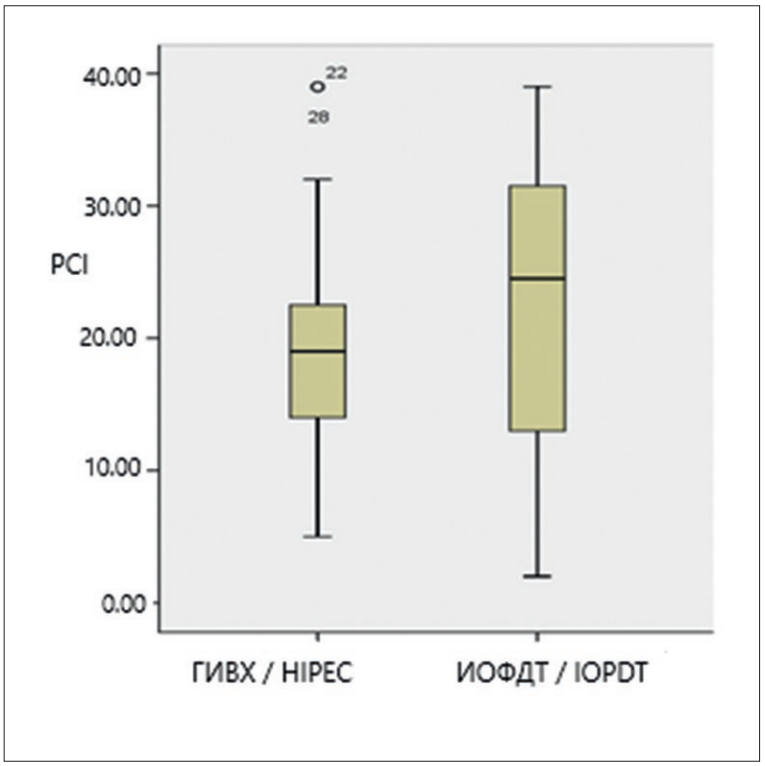

Рис. 1. Распределение больных по PCI
Fig. 1. Distribution of patients by peritoneal carcinomatosis index

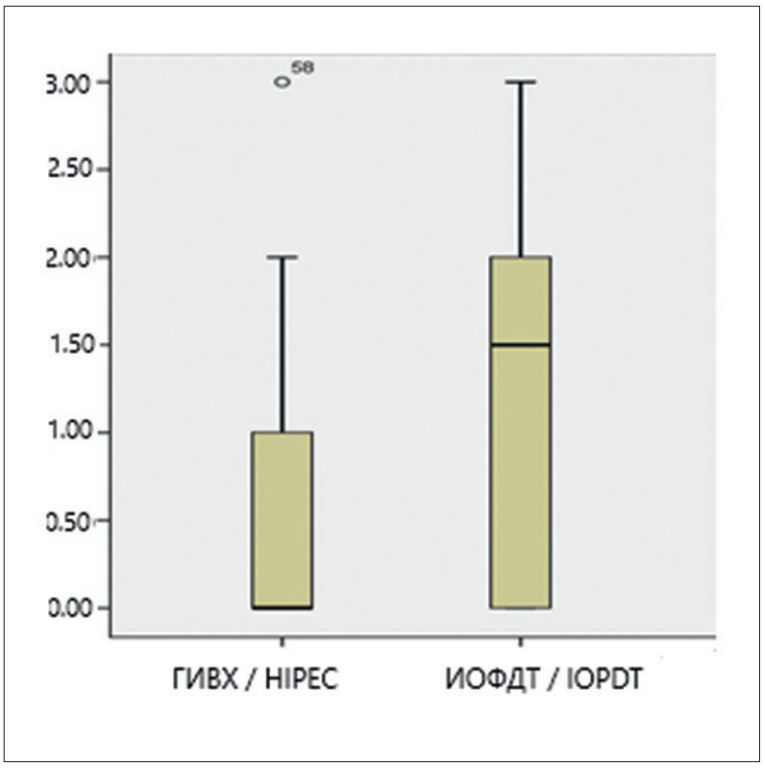

Рис. 2. Распределение больных по полноте выполненных циторедуктивных операций
Fig. 2. Distribution of patients according to the completeness of performed cytoreductive operations

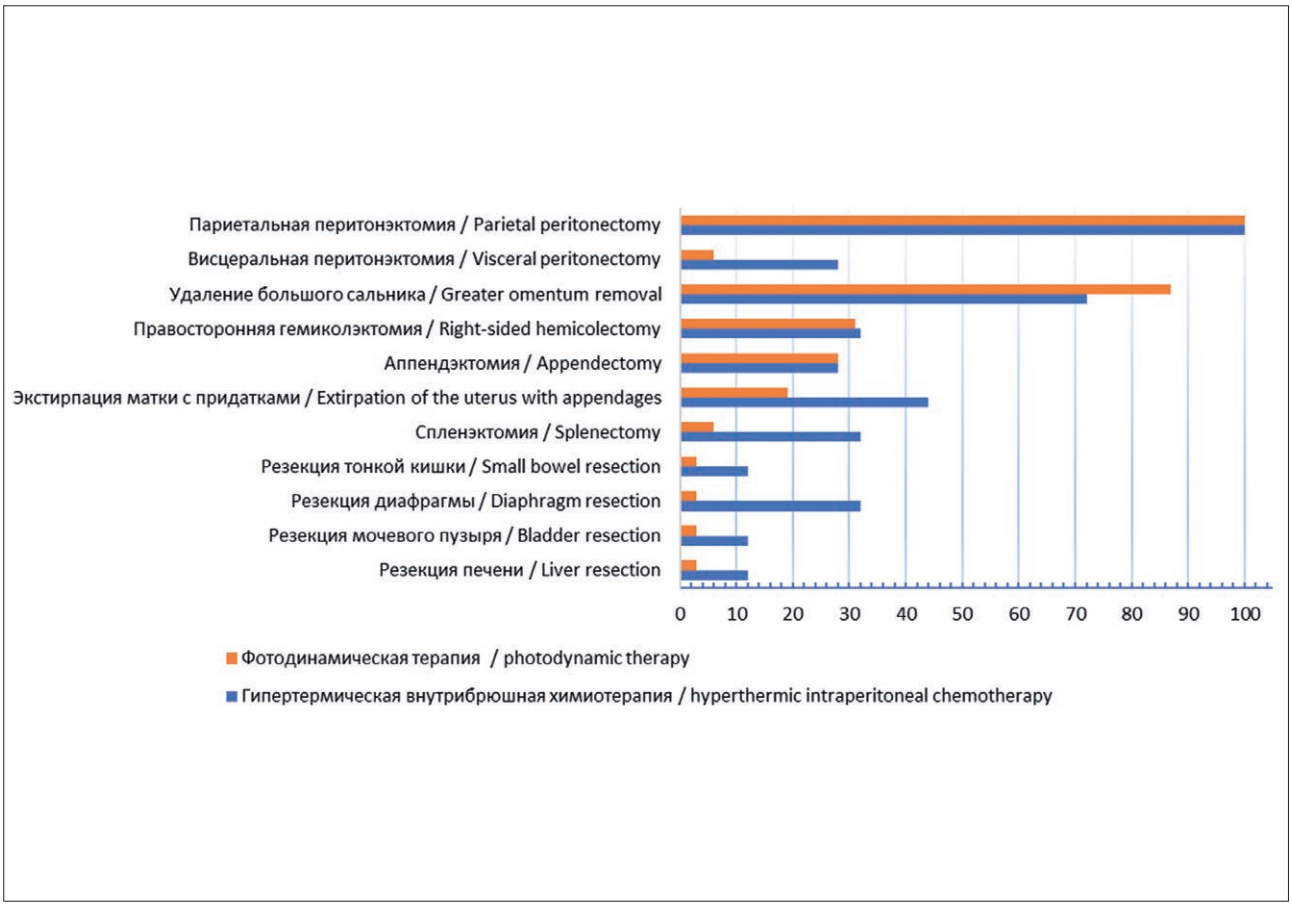

Рис. 3. Распределение больных по объему операции
Fig. 3. Distribution of patients by volume of surgery

Results

Immediate results

The average PCI value in the general group of patients was 20.75±10.6. In the group which underwent IOFDT, it was higher, 22±10.5 points, whereas in the HIPEC group it was 19.0±8.9 points. A comparative assessment by the Mann-Whitney U-test revealed no significant difference in the prevalence of the process (p=0.171) (Fig. 1).

The quality of the operation was evaluated by the Complete Cytoreduction Score. Optimal cytoreduction (CC0-1) was achieved in 40 patients (70.2%), and in the remaining 17 patients (29.8%), the residual tumor ranged from 2.5 mm to 2.5 cm or more. When analyzing the quality of surgery in patients with IOFDT, it was found that the number of patients with optimal and non-optimal (CC2-3) cytoreduction is approximately equal: 18 (51.4%) and 17 (48.6%), respectively. A completely different ratio was obtained in the group which underwent HIPEC. Optimal cytoreduction was achieved in 21 (91.3%) patients, in which 18 (85.7%) had no residual tumor visualized (CC0), and the rest of the patients had tumor dimensions not exceeding 2.5 mm (CC1). Suboptimal cytoreduction (CC2) was observed in only 4 patients (8.7%). A comparative assessment shows that a significantly higher number of optimal cytoreduction (p=0.002) was obtained in the group with intraoperative HIPEC (Fig. 2).

A comparative analysis of the performed scopes of surgery in the studied groups was carried out. All patients underwent subtotal parietal peritonectomy. Partial removal of the visceral layer is almost 5 times more often performed in patients with HIPEC: 28% versus 6.3% in the group with IOFDT. If the number of resections or extirpations of the large and small omentum (72% and 87.5%), hemicolectomies (32% and 31.2%), and appendectomies (28% and 28.1%) in the compared groups is similar, a clear tendency is observed concerning the higher number of surgical interventions in HIPEC group for the extirpation of the uterus with appendages (44% vs. 18.7%), splenectomy (32% vs. 6.3%), resection of the diaphragm (32% vs. 3.1%), resection of the bladder, liver, and small intestine (12% vs. 3.1%) (Fig. 3).

Complications during treatment were detected in 9 (15.7%) patients, including 3 in the IOFDT group and 6 in the HIPEC group. The hospital mortality rate

was 5.2%, all the deaths were in the HIPEC group. The characteristics of postoperative complications justify the choice of cytoreductive surgery as a treatment method, its volume, and the combination of intraoperative antitumor method. In the general group with optimal cytoreduction, complications were observed in 14.6%, and with CC2–CC3 cytoreduction completeness, in 20.8%. However, in patients who underwent IOFDT and achieved optimal cytoreduction, the complication rate was 11.1%, whereas in the group with HIPEC, the complication rate was more than twice as high (23.8%).

The analysis of the severity of complications in the general group of patients showed that Clavien-Dindo grade 2 was observed in 4 cases (IOFDT=2, HIPEC=2), 3a in 2 cases (IOFDT=1, HIPEC=1), 4a in 4 cases (all HIPEC), 4b in 4 cases (all HIPEC) and death (grade 5) occurred in 3 patients (all HIPEC). The data take into account the combination of these complications in 9 patients. The most frequent complication was gastrostasis, which was registered in 6 patients and was obviously associated with the removal of the omentum completely replaced by the tumor. In 2 cases, suppuration of the postoperative wound and perforation of the small intestine were revealed. In the comparative analysis, the complication rate in the HIPEC group was 24%, and the IOFDT group, 9.4%. All 3 fatal cases occurred in the HIPEC group, where the mortality rate was 12%. The main cause of death was multiple organ failure, its triggers being different: ileo-transverso-anastomosis failure, pancreatic necrosis, and multiple prolonged perforations of the small intestine. The patients underwent repeated relaparotomies, but the lethal outcome was not avoided. It is noteworthy that their PCI was in the range of 20-24 points, but it was necessary to achieve optimal cytoreduction (CC1 in 2 cases, CC0 in 1 case) to perform HIPEC. There were no lethal outcomes in the group with IOFDT.

Long-term results

The overall 5-year survival rate of patients in the HIPEC group was 86.6%. The median survival rate in the group of patients who were administered photodynamic therapy as an additional intraoperative method was 66 months, and the 5-year survival rate was 65.2%.

Discussion

The gold standard of treatment for patients with peritoneum pseudomyxoma is considered to be cytoreductive surgery with HIPEC. 5-year survival rates range from 62 to 92.5%, with a complication rate of 25-54.8% and a mortality rate of 2-9% [9-11]. However, these are the results in a carefully selected group of patients. In the actual practice, when there are only a few centers providing surgical treatment of carcinomatosis in Russia, and the number of patients, despite the extremely low incidence, is still high, we should not limit ourselves to considering the treatment tactics for such a small group. Another important point is the contraindications to the use of hyperthermic chemotherapy. General restrictions include age over 70, urinary system function issues, respiratory failure; local restrictions include the need to perform optimal cytoreduction. The penetration depth of the cytostatic agent, even at 42-43°C, is limited to 3 mm. Leaving a larger residual tumor, especially in the case of CC3, makes this very aggressive procedure useless. There are no approved standards for the treatment of patients with peritoneum pseudomyxoma, so a personalized treatment plan is selected for each patient. The surgery must be carefully planned. CT-PCI or ultrasound PCI is used to determine the prevalence of the process, the involvement of the serosa of the small and large intestine and their mesentery, the hepatoduodenal ligament, and the omentum sac. Based on the accumulated experience, we believe that with a PCI above 20 points it is not practical to plan cytoreduction with HIPEC, since an attempt to achieve optimal completeness of cytoreduction is not only questionable, but may also lead to the development of fatal complications. The use of IOFDT as an antitumor agent is limited by the need for exposure of the photosensitizer for 4 to 48 hours and the complexity of the effect on the visceral peritoneum. However, there are studies proving that the dose absorbed during the surgical stage of treatment from the operating theatre lamps, i. e., over at least 4 hours, corresponds to that of laser irradiation, and the treatment in this case is performed for both peritoneal layers [12]. In addition, the analysis of postoperative complications confirmed the safety of this technique and the fact that there are nearly no contraindications to its use. And the long-term results obtained, which take into account the initial PCI values and significantly worse cytoreduction indicators in the IOFDT group, make the IOFDT method more preferable in the clinical situation described due to the significantly lower frequency of post-operative complications and the absence of mortality.

Conclusion

The philosophy of treating patients with peritoneum pseudomyxoma involves maximum possible removal of tumor tissue from the abdominal cavity. Given the absence of hematogenic and lymphogenic metastasis in this disease, it is local control that determines the patient's prognosis and quality of life. The limited possibilities of systemic chemotherapy for mucinous neoplasia lead to surgical aggression and radicalism, however, the effectiveness of the

treatment depends on a careful choice of the happy middle ground based on a reasonable balance between the possible completeness of cytoreduction and the quality of life.

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PHOTODYNAMIC THERAPY IN THE TREATMENT OF INTRAEPITHELIAL NEOPLASIA OF THE CERVIX, VULVA AND VAGINA

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Abstract

In the present review the authors analyzed the effectiveness of treatment of intraepithelial neoplasia I-II-III of the cervix (CIN), vulva (VIN) and vagina (VaIN) using photodynamic therapy (PDT). PDT is a method based on exposure to light after preliminary introduction of a photosensitizer into the body with the formation of singlet oxygen, which has a cytotoxic effect. The results of research on the use of PDT with various photosensitizers in the complex of therapeutic measures in patients with CIN, VIN, VaIN are presented. These data on the effectiveness and safety of PDT, ease of use allow this medical technology to be attributed to one of the most promising areas in the treatment of pathological intraepithelial changes of the cervix, vulva and vagina. The presented information allows focusing the attention on the PDT method and informing doctors and researchers about the broad prospects for applying this treatment method in clinical practice.

Keywords: photodynamic therapy, photosensitizer, cervical intraepithelial neoplasia, vaginal intraepithelial neoplasia, vulvar intraepithelial neoplasia.

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ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ В ЛЕЧЕНИИ ИНТРАЭПИТЕЛИАЛЬНЫХ НЕОПЛАЗИЙ ШЕЙКИ МАТКИ, ВУЛЬВЫ И ВЛАГАЛИЩА

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Резюме

В обзоре литературы представлен анализ эффективности лечения интраэпителиальной неоплазии I-II-III степени шейки матки (CIN), вульвы (VIN) и влагалища (VaIN) с использованием фотодинамической терапии (ФДТ). ФДТ – метод, основанный на воздействии светом после предварительного введения в организм фотосенсибилизатора с образованием синглетного кислорода, оказывающего цитотоксический эффект. Представлены результаты исследований по использованию ФДТ с различными фотосенсибилизаторами в комплексе лечебных мероприятий у больных с CIN, VIN, VaIN. Приведенные данные об эффективности и безопасности ФДТ, простота применения позволяют данную медицинскую технологию отнести к числу наиболее перспективных направлений в лечении различ-

ной степени выраженности интраэпителиальных изменений шейки матки, вульвы и влагалища. Представленная информация позволит акцентировать внимание на ФДТ и информировать врачей и научных сотрудников о широких перспективах применения данного метода в клинической практике.

Ключевые слова: фотодинамическая терапия, фотосенсибилизатор, цервикальная интраэпителиальная неоплазия, влагалищная интраэпителиальная неоплазия, вульварная интраэпителиальная неоплазия.

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As doctors are trying to deal with the difficult, but solvable task of reducing cancer morbidity and mortality, the requirements for the selection of modern optimal methods of patient treatment are becoming more rigorous. The achievements in photochemistry, photobiology, and quantum physics have made possible the advances in this direction that provide alternatives to the traditional methods of treatment; a method worth focusing on among the new ones is photodynamic therapy (PDT).

The PDT mechanism is based on the ability of some photosensitizers (PSs) to accumulate in the tumor tissue and, when interacting with light radiation of a certain wavelength, initiate the formation of singlet oxygen through a series of photochemical processes, which has a destructive effect on the vital structures of tumor cells, leading to their death.- The damaging mechanism of photodynamic effects on tumor tissues is mainly determined by the average intracellular concentration of PS, its localization in the cell, and its photochemical activity: the quantum yield of singlet oxygen or free radicals generation [1, 2, 3, 4].

In addition to the direct phototoxic effect on tumor cells, an important role in the destruction mechanism is played by impaired blood circulation in the tumor tissue due to damage to blood vessels endothelium [5], a hyperthermic effect associated with active light absorption by tumor cells, as well as cytokine reactions caused by stimulation of the tumor necrosis factor and activation of macrophages, leukocytes, and lymphocytes [6, 7].

The increased interest in PDT is caused by the wide possibilities of using light radiation in oncology and PDT's advantages over other methods of treatment, such as low invasiveness; selectivity of destruction of malignant tumors and pathological areas; minimal damage to healthy tissue; the possibility of repeated use, as no resistance develops after repeated cycles; the absence of toxic and immunosuppressive reactions [8].

In oncogynecology, the method can be used for both radical and palliative treatment, which determines its use in women with precancerous and neoplastic diseases of genital organs [9].

The increasing attention of gynecologists and oncogynecologists to PDT is associated with the need to expand the range of organ preservation treatment options, which is extremely important in connection with the steady increase in the incidence of cervical, vulvar, and vaginal cancer in young women over the past two decades [10]. This method meets the main characteristics of an organ-preserving treatment, i.e., high effectiveness against the pathology, low frequency of relapses after treatment, tolerability (minimal number of side effects), and ease of administration of the treatment [11]. However, with the advent of the viral concept of cervical cancer, and with frequent detection of human papillomavirus (HPV) in cervical intraepithelial neoplasia (CIN) cases in direct proportional to the severity of the changes (CIN I: 25%, CIN II: 80%, CIN III: 88–100%) [12], the identification of the viral genome in intraepithelial neoplasias of the vagina (VaIN) and vulva (VIN), respectively, in 80% and 20% of cases [13], etiopathogenetic therapy becomes the center of attention, as it is aimed not only at the pathological process itself, but also at HPV eradication.

To date, there are no clear recommendations in the world for the treatment of HPV-associated intraepithelial pathology of the cervix, vulva, and vagina, which may have various degrees of severity. It has become possible to revise the traditional treatment approaches, taking into account the need for eradication of papillomavirus infection (PVI), thanks to the accumulated experience and modern developments in the field of medical technologies.

The specifics of the HPV life cycle are one of the determining factors of the antiviral treatment effectiveness. In the episomal form of HPV, physical methods of treatment aimed at destroying the primary lesion or immunotherapy are successfully used, provided that the duration of PVI is not more than one year and no virus persistence is present [14]. When HPV is integrated into

the genome of the host cell, interferon therapy is powerless, since the infected cells contain no virus as such in the traditional sense of the word, and, therefore, treatment methods aimed at destroying viral heterotopias are necessary [15].

PDT and CIN

The methods of treatment of CIN I–II–III and microinvasive cervical cancer (1A1 st.) based on ablation (diathermic and radiocoagulation, cryodestruction, laser vaporization) and removal (laser, electric, radio, knife excision/conization) of the primary lesion as a monotherapy are not effective enough for the elimination of HPV, as the effect is directed only on clinical lesions without affecting multifocal areas with latent or subclinical form of PVI, insufficient depth of destruction of the epithelial layer to the basal layer, where the reservoir of papillomaviruses is located, the absence of targeted destruction of papillomaviruses and irradiation of the transition zone and the cervical canal, which together lead to the persistence of the viral genome [16, 17, 18, 19]. The expression of HPV DNA at the sites of cervical primary lesions, the necrosis zone, the mucous membranes adjacent to the edge of destruction or resection, and the reactivation of the viral genome together provoke a high risk of CIN relapses in a fairly short period of time (from 12 to 36 months), the progression of the tumor process to preinvasive or eventually to microinvasive cancer (from 15 to 50%) and low effectiveness of treatment (45– 97%) [19, 20].

The potential of PDT in the treatment of cervical cancer pathology has been studied since the 1990s. In oncogynecology, the choice of the cervix as the first model for clinical research is explained by the relevance of the outcomes, and the development and implementation of new approaches to the prevention and treatment of cervical cancer. The incidence of the disease has been growing in a number of countries over the past two decades. Another reason is the availability of visual and non-invasive research methods. Some studies examined the issues related to the pharmacokinetics of exogenous and endogenous PS chemicals, the development of scientifically based methodological approaches to PDT with the use of Russian-made pharmaceuticals of various groups in achieving the antitumor and antiviral effect, depending on the severity of intraepithelial cervical changes: laser irradiation modes; the choice of PS and methods of administration (local, systemic); calculation of the light dose and the method of radiation delivery to abnormal tissues [21, 22].

The results of the use of PDT in the treatment of cervical tumor pathology were first published in 1996. In the study, attempts were made to optimize PDT regimens in the treatment of non-invasive cervical cancer with intravenous administration of photofrin in order to achieve complete regression. A positive effect was registered in 62% of clinical observations at a light dose of 100–140 J/cm$^{2.}$ PDT of the cervix with non-invasive tumor changes is decribed as an organ-sparing treatment method [23].

Promising results of PDT in the treatment of CIN I–II–III were obtained with topical application of aminolevulinic acid hexyl ether (HAL) and the use of a light dose of 100 J/cm^2 and a laser wavelength of 633 nm. The studies related to the pharmacokinetics and selectivity of porphyrins established the optimal concentration of the drug (10 mmol/l) and the exposure time (5–9 h). This methodological approach made it possible to achieve a complete regression in 63% cases, with the best treatment results obtained in cases with minimal cervical changes: 100% for CIN I, 50% for CIN II, and 43% for CIN III [24].

PDT with photohem at a dose of 2 mg per kg of body weight with intravenous administration, a light dose of 100–150 J/cm^2, and exposure time of 24 h led to complete regression of CIN II–III in 93% of cases, which indicates the method's high effectiveness in antitumor treatment. Photoreduction with photohem is proposed as a fertility-preserving method, an alternative to destructive treatment of the cervix [25].

The prospects of PDT in the treatment of CIN II–III in comparison with traditional methods were demonstrated with photolon (0.5% aqueous solution), leading to complete regression of cervical pathology in 65% of women, with intravenous administration of the drug, an exposure time of 2.5 hours, laser radiation wavelength of 662 nm and radiation power density of 100–300 W/cm$^{2.}$ The authors found that errors in the calculation of the light dose, which is particularly important in achieving maximum photodynamic destruction, can lead to a lack of response to treatment, or, on the contrary, to pronounced destructive changes in the tissues in the PDT area, which in turn leads to prolonged wound healing, rough scarring, and longer rehabilitation periods [26].

Another study conducted in the framework of phase III clinical trials of radachlorin, a photosensitizer produced in the Russian Federation, describes the results of PDT in patients with precancerous conditions and initial cervical cancer. The study included 30 patients: 4 with cervical ectopia, 5 with CIN II, 13 with CIN III, 4 with carcinoma in situ, and 4 with stage Ia cervical cancer. The PDT session involved irradiation of the entire cervical canal and the vaginal portion of the cervix. In the groups diagnosed with cervical ectopia, CIN II, and carcinoma in situ, complete regression was registered in all cases. In the group with CIN III, complete regression was obtained in 77% of cases, partial regression, in 23%; in the group with a diagnosis of stage Ia cervical cancer, in 75% and 25%, respectively. All patients with partial regression underwent a second course of PDT, which had a full effect [27].

The ability of PDT to provide not only a pronounced antitumor effect but also antiviral effect as an independent method of treatment has recently attracted significant attention to the method. One of the first publications on this subject was a study performed on a large clinical material in women with HPV-associated precancerous and initial tumor changes in the cervix; treatment was performed with photohem (intravenous administration, dose: 3.0 mg per kilo of body weight, exposure time: 48 h, wavelength: 630 nm, energy density: 150–200 J/cm^2, power: 150–250 MW/cm^2) and photosense (intravenous administration, dose: 0.3 mg per kg of body weight, exposure time: 24 h, wavelength: 675 nm, energy density: 100–150 J/cm^2, power: 150–250 MW/cm^2) [28]. The difference between the developed PDT technique and other variants of photodynamic destruction of the cervix is the use of polypositional irradiation of the cervical canal throughout the entire area of the vaginal portion of the cervix. The authors believe that this approach makes it possible to selectively destroy the foci of precancerous and initial tumor pathology in 90–92.5% and 77.7–80.7%, respectively, and to achieve an antiviral effect due to the destruction of clinical, latent and subclinical forms of PVI in 94.2% of cases. Based on the results of the study, it is possible to see PDT as the minimalistic option of organ-sparing treatment, which is of great interest in the light of the proven etiological role of HPV in the development of cervical cancer.

The antiviral effect of PDT has created a new direction towards solving the urgent problem of preventing relapses of HPV-associated CIN and cervical cancer. The treatment method that consists of local application of a Russian-made pharmaceutical product based on 5-aminolevulinic acid (5-ALA), an inducer of the synthesis of protoporphyrin IX, an endogenous photosensitizer (dose: 0.1 mg/cm^2, exposure time: 6 h, wavelength: 635 nm, energy density: 150 J/cm^2, power density: 150–250 MW/cm^2), allows achieving complete eradication of PVI after primary organ-preserving treatment in 96% of cases. HPV elimination is caused by irradiation of the remaining part of the cervical canal and the vaginal portion of the cervical stump after conization with the polypositional effect of light energy on the resection zone, the exposure involving the vaginal arches. PDT is recommended for antiviral purposes at the second stage of treatment after knife amputation, laser, electric and radiowave cone excision/conization of the cervix, as well as as an alternative to diathermal and radiocoagulation, cryodestruction, and laser vaporization in the treatment of cervical PVI [29].

A number of studies in subsequent years evaluated the antitumor and antiviral efficacy of PDT in the treatment of CIN I–II–III with photosensitizers of various groups.

M. C. Choi et al. (2013), in their study where they used photohem as a PS in patients with HPV+ CIN II–III (intravenous administration, dose: 3.0 mg per kg of body weight, exposure time: 48 h, wavelength: 630 nm, energy density: 150 J/cm^2), complete regression was observed in 98.1%, and HPV eradication in 89.8% of the cases [30].

P. Hillemanns et al. (2014), in PDT of CIN II, used hexyl aminolevulinate in various concentrations (5%, 1%, and 0.2%) as an ointment application on ectocervix (exposure time 5 h, radiation wavelength 629 nm, energy density 100 J/cm^2). To assess the antitumor effect of treatment, a cervical biopsy was performed 3 and 6 months after the treatment. The best results in terms of CIN II complete regression were found in observations with a 5% concentration of the drug (95%), in other cases, this indicator was 79% and did not depend on the concentration. The best results in terms of complete eradication of HPV were registered in cases of 5% ointment application (83%), with the use of lower concentrations of the product (1% and 0.1%), complete eradication of HPV was noted only in 48% and 42% of cases, respectively. Thus, the antiviral effectiveness of hexyl aminolevulinate is directly dependent on the concentration of the product. The index of viral elimination is less high in comparison with the data published by other researchers, which may well be due to the absence of photo-radiation of the cervical canal [31].

Y. K. Park et al. (2016) also indicate high antitumor and antiviral efficacy of PDT with photofrin (intravenous administration, dose: 2 mg per kg of body weight, exposure time: 5 h, radiation wavelength: 629 nm, energy density: 100 J/cm^2) in the treatment of CIN II–III. Photodestruction was performed on both exocervix and endocervix. Complete regression of intraepithelial pathologic changes and HPV elimination were achieved in 95 and 90.9%, respectively [32].

H. Cai et al. (2020) report a complete regression of CIN I–II–III in 100% of cases, while HPV eradication was observed in 83.3% of cases after 4–7 PDT sessions with 5-ALA in the form of 20% ointment application on exocervix (exposure time: 3 h, radiation wavelength: 635 nm, energy density: 100 J/cm^2, power density: 80 MW/cm^2). The effect of the treatment was evaluated after 6–7 months by a loop electrosurgical excision. The results of the study indicate the need for endocervix irradiation in order to achieve a pronounced antiviral effect [33].

PDT and VIN

Publications in the Russian and foreign literature devoted to PDT in background diseases, precancerous conditions, and initial vulvar cancer are few and limited to a small number of clinical observations. Early detection and treatment of diseases of this localization is still one of the most pressing issues of gynecology and oncogynecology. The possibilities to prevent the development of a tu-

mor and cure pre-cancerous changes in the tissues exist, which urges active research for new effective treatment methods and their further development. In the past, vulvar intraepithelial neoplasia (VIN) was considered a pathology of women aged over 40, but in recent years, the condition is increasingly more often diagnosed in younger women (aged 25–40). A direct correlation was established between the presence of types 16 and 18 HPV in vulvar tissues and VIN in young women [34, 35].

Treatment options for dystrophic diseases of the vulva are extremely diverse, which is explained by their low effectiveness, as well as by the recurrent nature of the disease. The methods of treatment include conservative and surgical ones. The first treatment option includes hormone therapy, but it should be noted that it does not actually cure but only eliminates the symptoms of the disease and promotes a temporary remission. Surgical methods include cryodestruction, laser vaporization, and surgical excision of lesions. Unfortunately, the relapse rate after such treatment is high, as well as scar tissue deformity, which forces doctors to look for new therapies that combine the optimal therapeutic effect and the absence of complications [36]. One of the latest approaches to the vulvar pathology treatment is PDT. The potential of the method in the treatment of VIN is due to its non-invasive nature and its wide applicability [37, 38].

In one of the first studies that evaluated the effectiveness of PDT in the treatment of background and precancerous diseases of the vulva with the use of 5-ALA in the form of 20% ointment application, the following treatment modes were used: exposure time: 6 h, radiation wavelength: 629 nm, energy density: 100 J/cm^2 power density: 100–200 MW/cm$^{2.}$ Patients with dystrophic changes of the vulva (lichen sclerosis, squamous cell hyperplasia) were administered two courses of PDT with an interval of 30 days, while patients with VIN I–III had 2– 3 courses of treatment. In the group of women with lichen sclerosis, the positive effect of treatment, established on the basis of the disappearance of itching and dryness, and the achievement of more elasticity and softness of the skin on the labia majora, was noted in 85% of patients. Complete regression of hyperplastic processes of the vulva was diagnosed in 100% of cases, the outcome of treatment remained after 1, 3 and 6 months. High efficiency of PDT was observed in the vulva irradiation with VIN I–II–III phenomena. Control histological examinations performed after 1 month in all cases with VIN I–II did not reveal epithelial atypia, whereas in 60% of women with VIN III, the condition regressed to VIN I–II, which was regarded as an indication for another course of PDT; such course then demonstrated complete effectiveness. No relapses of the disease during one year after the treatment were noted, and a good cosmetic effect was achieved, which is of great importance for young and middle-aged patients [39].

In another study, in which 50 patients with vulvar leukoplakia underwent PDT with photolon (intravenous administration, dose 1,8–2.5 mg per kg of body weight, exposure time: 2.5–3 h, radiation wavelength: 661 nm, energy density: 30–100 J/cm^2, radiation power density: 100–170 MW/cm^2) a high cure rate, 92%, was established after 3 months. The results of the study allow us to make conclusions on the possibility of the use of this methodological approach in the treatment of background diseases of the vulva to achieve satisfactory functional and cosmetic results [40].

A similar satisfactory effect from treatment was registered after 3 courses of PDT with 5-ALA (20% ointment, exposure time: 3 h, radiation wavelength: 633 nm, energy density: 120 J/cm^2 power density: 100 MW/cm^2) in the treatment of VIN III. Complete regression for 12 months was established in 94%, and a good cosmetic result was observed in 71% of cases. A significant improvement in the quality of life and mental health was observed in all patients [41].

The lower effectiveness of PDT with 5-ALA in the treatment of VIN II–III is indicated in three studies. In two of them, when applied topically, as 10 g of ointment (exposure time: 2.6 h, radiation wavelength: 635 nm, energy density: 116 J/cm^2 (+/- 16 J/cm^2), power density: 100 MW/cm^2 (88 +/-17 MW/cm^2) and 10 ml of 20% ointment (exposure time: 3 h, radiation wavelength: 635 nm, energy density: 100 J/cm^2 power density: 100 MW/cm^2). complete regression over a short observation period of 8 weeks and 12 weeks, respectively, was established, in 57% [42], and 52% of cases [43]. In the third study, when 5-ALA was used in the form of a patch (38 mg/cm^2, exposure time: 20–28 minutes, radiation wavelength: 630 nm, energy density: 100 J/cm^2), a positive effect of PDT was registered in 38% of observations [44].

PDT and VaIN

The PDT method in the treatment of VaIN is in its initial stage, and, therefore, there are no clear recommendations for its widespread implementation in clinical practice. At the moment, there are only a few studies describing this technique, but the sample of patients is too small for the results to be convincing [45].

In a combined study aimed at evaluating the effectiveness of PDT in the treatment of VIN II–III and VIN II–III with the use of photohem at a dose of 2 mg per kg of body weight (exposure time: 48 h, radiation wavelength: 630 nm, energy density: 150 J/cm^2), complete regression was established in 3 out of 5 patients after 3 months. In 2 patients, a partial effect was observed, in connection with which one patient underwent a vulvectomy, and the other underwent a second course of PDT with 5-ALA in the form of ointment application. In two cases with complete regression, there was a relapse of VIN III and VIN II one year after the treatment. Cutaneous phototoxicity

and facial edema, which was regarded as a side effect of treatment, were observed in one patient. The authors concluded that PDT is effective as an alternative method of treating precancerous lesions of the lower parts of the female genital organs, with normal anatomy and sexual function of the organs maintained [46].

Another study published in 2020 compared the effectiveness of combined treatment (CO2 laser + PDT) and CO2 laser monotherapy in the treatment of 40 patients with HPV-associated VAIN I. In the first group (n=20), treatment was carried out with one course of CO2 laser and three courses of PDT with 5-ALA (topical 20% gel, exposure time: 3 h, laser wavelength: 635 nm, energy density: 100–150 J/cm^2, interval: 1 week). In the second group (n=20), 3 procedures were performed with a CO2 laser. In the group of patients who received combined treatment, complete regression was 85%, the frequency of complete eradication of HPV was – 95%, whereas in the second group it was – 65% and 25%, respectively. The data obtained suggest that the inclusion of PDT in the treatment of HPV-associated VaIN I improves the outcome in terms of complete regression and eradication of the viral genome. The method is safe and well-tolerated by the patients [47].

Thus, the analysis of the presented PDT results showed their high practical significance. It is important to emphasize the following facts.

PDT for precancers and primary cancers of the cervix, vulva, and vagina is the minimalist option of organ-sparing treatment, an alternative to surgical methods with the preservation of the anatomical and functional integrity of the organs, which is important for the implementation of reproductive function.

The results of the antiviral efficacy of PDT are of great interest in connection with the proven etiological role of HPV in cervical carcinogenesis and the frequency of PVI presence in female genital organs. The relationship between the complete regression of the tumor process and the eradication of PVI is obvious, which indicates the preventive value of photodynamic radiation against the development of relapses of the disease. A plausible explanation of higher rates of eradication of oncogenic HPV types in comparison with other methods of treatment, and the absence of reinfection over a long period of observation, can be based on selective accumulation of photosensitizer in infected cells, their direct phototoxic destruction, as well as point-like effects on clinical, latent, and subclinical foci of multifocal viral damage. This approach leads to a significant reduction in the likelihood of HPV-associated relapses, reducing the duration of antiviral therapy in comparison with standard therapeutic methods of treatment.

The reasons for the ineffectiveness of PDT are noncompliance with the developed and approved PDT methods for each nosological form of the disease.

PDT provides full-fledged medical and social rehabilitation of patients, and the economic significance of the method comes from the fact that no labor rehabilitation is required after the treatment.

PDT can be considered as a secondary method for prevention of HPV$^+$ CC, HPV$^+$ vulvar cancer, HPV$^+$ vaginal cancer, but PDT can also be used as an independent treatment method, which opens up broad prospects for its use in gynecology and oncogynecology.

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