Ultraviolet A/blue light-emitting diode therapy for vulvovaginal candidiasis: a case presentation

Mariana Robatto[1,2] · Maria Clara Pavie[1,2] · Igor Garcia[1] · Manoela Porto Menezes[1,2] · Milena Bastos[1] · Handerson Jorge Dourado Leite[1] · Andreia Noites[3] · Patrícia Lordelo[1,2]

[1] Bahiana School of Medicine and Public Health, Av. Dom Joao VI, 275, Brotas, Salvador, Bahia 40290-000, Brazil

[2] Center for Care of Pelvic Floor, Salvador, Bahia, Brazil

[3] School of Allied Health Technologies, Polytechnic Institute of Porto, Porto, Portugal

Abstract

Vulvovaginal candidiasis (VVC) is a frequent infection of the female genitourinary tract. It is considered the second most common genital infection in women, after bacterial vaginosis. VVC is treated with oral or topical azole derivatives. However, these agents may lead to adverse reactions and their chronic use might lead to resistance to antifungal agents. Given that the ultraviolet A/blue light-emitting diode (LED) is an electromagnetic radiation source with antimicrobial properties, it is hypothesized that this resource may be a non-drug alternative to the treatment of vulvovaginitis. A technical/experimental safety test was conducted to characterize the light source spectrum and temperature generation of the device, followed by a pilot study in a 52-year-old patient with a clinical diagnosis of VVC confirmed by culture and examination of fresh vaginal samples, owing to the presence of lumpy vaginal discharge and a complaint of pruritus. The vulva and vagina were exposed to 401 ± 5 nm ultraviolet A/blue LED irradiation in a single session, divided into two applications. A reassessment was performed 21 days after the treatment. The light-emitting device had a visible spectrum, in the violet and blue ranges, and a maximum temperature increase of 7 °C. During the reassessment, the culture was found to be negative for fungus, and the signs and symptoms of the patient had disappeared. A light-emitting device with a spectrum in the range of 401 ± 5 nm could potentially be an alternative treatment modality for women with VVC, as it led to the resolution of clinical and microbiological problems in our patient.

Keywords Vulvovaginal candidiasis · Women · Phototherapy

Introduction

Vulvovaginal candidiasis (VVC) is an infection of the vulva and vagina caused by the abnormal growth of several *Candida* species [1, 2]. This pathology is one of the most common diagnoses in gynecological practice and the second most common genital infection [2]. Studies have shown that 15 to 25% of adult women present with fungal colonization despite being asymptomatic and that 75% of them will develop the disease at some point in their lives [2, 3].

The characteristic clinical manifestations include pain, hyperemia, and edema in the vulval and vaginal regions, in addition to pruritus and the presence of lumpy and whitish vaginal secretions [4, 5]. These signs and symptoms are not pathognomonic of VVC and the diagnosis must be confirmed by laboratory tests, such as microscopy with an examination of fresh vaginal samples and the 10% potassium hydroxide (KOH) test. This examination allows visualizing the presence of hyphae and/or fungal spores [5, 6].

The treatment of VVC involves the use of antifungal agents, either orally or topically, which resolves 80 to 90% of the cases [7]. However, the use of these agents can lead to the development of adverse reactions such as dysuria, pruritus, and gastrointestinal disorders. In addition, in cases of reinfections, resistance to antifungal agents may occur [8]. These adverse effects, in addition to the variation in the resolution rate, suggest the needs to continue searching for more cost-effective and non-drug therapies.

Accordingly, a hypothesis arises that the blue/violet light-emitting diode (LED) may be an alternative treatment for

women with VVC because of its antimicrobial effect that has been proven by several studies [9–11]. Moreover, it is considered a safe, non-invasive, painless, and non-toxic technique for use in several types of tissue [12, 13]. This resource consists of a semiconductor diode subjected to an electric current with a wavelength ranging from 400 to 470 nm, being the 405-nm light used in studies that aimed to test its fungicidal effect. To our knowledge, there is yet no standardized method for the use of this technique in terms of the application parameters for obtaining different effects and its use in the genital region, which is rarely mentioned in the literature [10, 13, 14].

Some reports have indicated that LED irradiation, more specifically that using the blue spectrum (400–470 nm), has a fungicidal effect. This is because fungi contain porphyrins, which are compounds that generate highly reactive oxygen species when in contact with light, consequently leading to cell death [9, 10]. Studies have reported that porphyrins absorb light more effectively at a wavelength of about 400 nm [10].

Therefore, with the knowledge that VVC is an infectious disease of the genitourinary tract that is common in women of reproductive age [1], and because of the shortage of non-drug therapies for this condition [15], we aimed to describe the use of ultraviolet A/blue LED with a wavelength of 401 ± 5 nm in a patient with a clinical manifestation of candidiasis. Before the treatment, a technical/experimental safety test was conducted to characterize the light source and temperature generation of the device.

Study design

This study was carried in two moments, an experimental (a technical study) and other pilot study precedes a randomized clinical trial in a woman with VVC.

Measurement of the light spectrum to characterize the light source

At first, a technical study was carried out in the laboratory of the Innovation Center of the Federal Institute of Bahia (IFBA) to verify the light spectrum and irradiation provided by the LED device developed for the study (patent application no. BR 10 2017 026980 9). The device has no serial number or mark and has three LED sources arranged in a circular area of 3.5 cm and adjusted for a single power. The three points of light, observed from a planar view, are parallel to each other, with 0.6 cm between them (Fig. 1).

The measurements were performed in a room, following the parameters of the National Institute of Standards and Technology (NIST) Special Publication 250-37 – Photométric Calibrations, NIST, 1997. An ultraviolet-visible spectrometer (registry/serial number USB2G48645, from Ocean Optics, model USB 2000) was used to characterize the light source.

The irradiance read by the spectrometer was 3.01 mW/cm^2. The reading was performed under conditions of total darkness, with a sensor indicating the reading "0" for all measurement ranges. The sensitive area of the sensor of the spectrometer was illuminated perpendicularly, 5 cm from the device that was irradiating the LED light, with vertical distribution (Fig. 2).

Measurement of the temperature generated by the ultraviolet A/blue LED light source of 401 ± 5 nm

At the time of measurement, the testing room had a temperature of 22.8 ± 0.6 °C and a relative humidity of 62.9 ± 9%. Measurements of the temperature generated by the device were performed in the power position, for a total time of 45 min, with a measurement interval of 5 s. The LED light source was suspended 3 cm from the temperature sensor by a support with three contact points (Fig. 3). A measurement equipment with a calibrated system, developed in the IFBA, was used for the temperature measurements.

Description of the use of the ultraviolet A/blue LED in a woman with VVC

In a second moment, the study was carried out from May to June 2017 and was approved by the Research Ethics Committee of the Federal University of Recôncavo Baiano (CAAE: 56391416.1.0000.0056). The clinical trial was registered at www.clinicaltrials.gov (registration no. NCT03075046).

The patient was a 52-year-old woman (married, menopausal, and undergoing hormonal therapy) who underwent a gynecological examination in the city of Salvador, Bahia, because of a complaint of lumpy and pruritic vaginal discharge in the external genitalia. She was seeking a non-drug alternative for the treatment of her symptoms.

After signing the informed consent form, the patient underwent anamnesis and a gynecological examination in the clinic, and the presence of lumpy discharge and vulvar erythema were confirmed. After the physical examination, cytological analysis with an examination of fresh vaginal samples was performed with a saline solution and 10% KOH, and the presence of fungal hyphae was identified. Then, assessments of vaginal pH and vaginal secretion (for the determination of the cervical-vaginal microflora and cytology), as well as culture-specific tests for fungi, were performed. The samples were collected and sent to a laboratory for clinical analysis, as authorized and accredited by the network of service providers under the laws of Brazil.

Fig. 1 Presentation of the LED source. Three sources of LED in planar presentation

The blue/ultraviolet led with a wavelength of 401 ± 5 nm and irradiance of 3.01 mW/cm^2 was used in a single session in two ways:

Initially, light was applied for 30 min (or 1800 s) within the vaginal canal using a disposable clear acrylic speculum (Fig. 4), which was 10-cm long (Fig. 5) and was used at its maximum aperture, allowing the vaginal canal diameter to increase by 9 cm (Fig. 6). In order to calculate the energy delivered in this application, absorption of speculum was considered negligible and the loss of light power was corrected as a function of the increase in distance, obeying the law of the inverse of the square of the distance. Thus, the light output delivered at 10 cm was calculated by the following ratio: $P1 = (d2/d1)^2 \times P2$; where P1 is the light power at 5 cm, d1 at 5 cm (or 0.05 m), d2 at 10 cm (or 0.1 m) and P2 at 10 cm. Thus, 3.01 mW/cm^2 = $(0.10/0.05)^2 \times P2$, then P2 = 0.752 mW/cm^2. The energy delivered for this application was calculated according to the equation: Energy (J) = Power (W) × time (s), logo: Energy = 0.752 mW/cm^2 × 1800 = 1.353 J/cm^2.

Immediately after, the light has been applied at a distance of 5 cm from the vulva. For this, the device was held in a holder for 30 min. The labia majora were separated and fixed in position with adhesive tape (Nexcare Micropore tape 25-mm wide) to better expose the inner region of the vulva and the vaginal introitus during LED irradiation (Fig. 7). Thus, the energy delivered for this application was: Energy = 3.01 mW/cm^2 × 1800 = 5.418 J/cm^2.

It is worth noting that the equipment did not contact with the tissues or secretions of the patient.

During the session, only the genital region of the patient was exposed, with the hip flexed and abducted, and the knees flexed. During the treatment, the physiotherapist who applied the light stayed in the room to assess the patient for any discomfort, sensation of temperature increase, and/or pain. If the patient reported any discomfort, the session would have stopped. In addition, the patient was advised to return for examination if she noticed worsening or persistent clinical manifestations or any adverse reaction to the treatment.

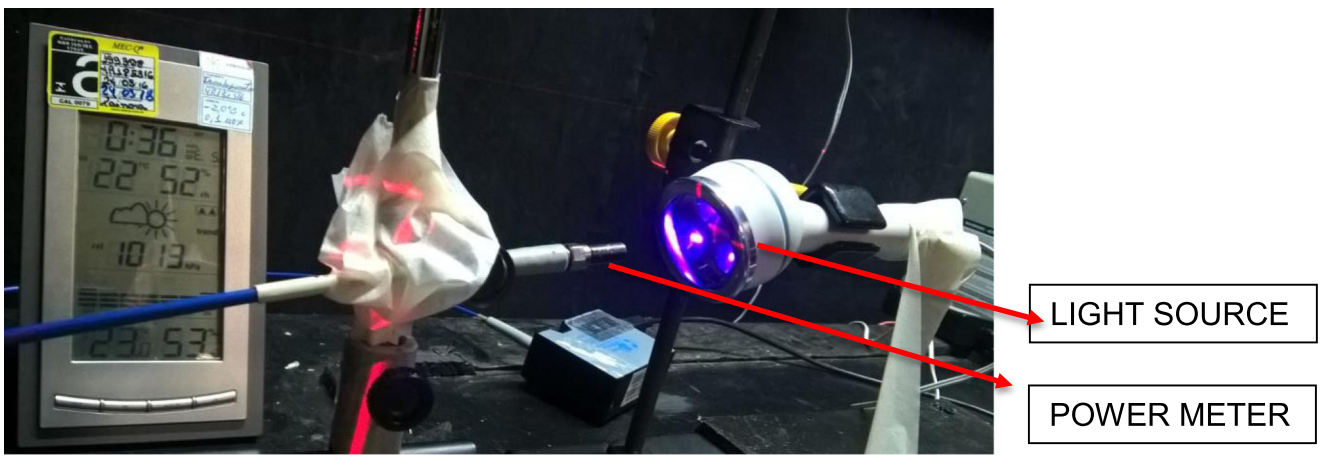

Fig. 2 Arrangement for characterization of the LED, measuring within 5 cm of the light source

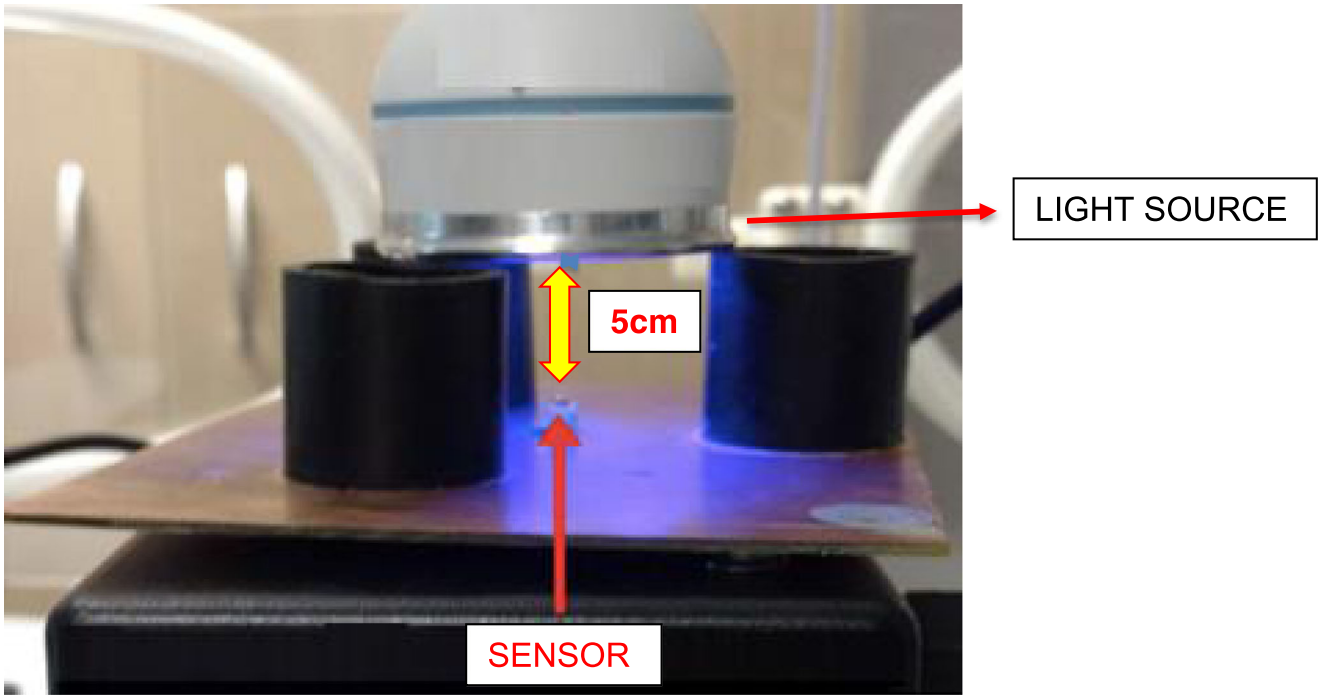

Fig. 3 Arrangement for temperature measurement. The temperature sensor was positioned 5 cm away from the light source

Pre- and post-test evaluation

Microbiological evaluation was performed using a fungus culture test with quantitative analysis of colony-forming units (CFUs). Secretions were collected before the LED application and 21 days after treatment completion.

In addition, vaginal pH and cervix-vaginal cytology and microflora were also assessed before therapy and 21 days after the completion of therapy. The pH measurement was performed in the vaginal canal for 1 min. The pH was measured according to the color change of the McolorpHast tape that was placed in the vaginal canal for 1 min. Microflora and cervico-vaginal cytology were assessed by collecting secretions and evaluating for cellular atypies, cell types present, and the absence or presence of inflammation.

The clinical manifestations of the patient were also evaluated before and 21 days after LED light application.

Results

The light source emits light in the ultraviolet A and visible (blue range) regions (Fig. 2). The wavelength of the light spectrum and wave peak value was 401 ± 5 nm at maximum power (Fig. 8). The results of the measurements at different times and device powers are shown in Fig. 9. In addition, an increase in temperature of approximately 7 °C was observed when testing the equipment in the laboratory with the maximum available power according to Fig. 9. The graph shows that the temperature does not increase significantly, even in an object of low specific heat (such as the temperature sensor) and also shows that the temperature tends to stabilize around 35 min.

The evaluation of the patient's microbiological status confirmed that she had *Candida* infection (with the growth of 80 CFUs) before the beginning of the treatment. Twenty-one days after the light application, the fungus culture showed an absence of growth. Thus, the patient was negative for fungi in the final report.

In the evaluations before the application of LED therapy and at 21 days after the treatment, it was observed that there was an increase in pH after treatment (pH 4.0 before treatment vs. pH 5.0 at 21 days after LED irradiation).

Cervical cytology did not show any cellular atypia at any time. However, in the microflora evaluation before treatment, benign reactive cellular alterations associated with moderate inflammation were observed, including the presence of *Candida* and *Lactobacillus*. Cytological examination performed 21 days after the end of treatment revealed discrete inflammation and the presence of only *Lactobacillus*.

Concerning the clinical condition, there was an absence of signs (lumpy discharge and vulvar erythema), and the patient reported that the symptoms of pruritus and burning stopped after the LED treatment. She was then followed up through quarterly telephone contact, over the course of 12 months, and remained without complaints during the period. Therefore, no new tests were requested.

Fig. 4 Application of LED 401 ± 5 nm in the vaginal region using a speculum

The patient did not report pain, sensation of a temperature increase, and discomfort during the LED treatment sessions. In addition, she did not report any adverse effects after treatment.

Discussion

The LED device was verified to have a wavelength in the range of 401 ± 5 nm, within the ultraviolet A and visible blue ranges. This evaluation demonstrated the degree of safety of ultraviolet A/blue LED, an innovative intervention using a prototype, as a non-drug treatment for vaginal candidiasis. The lack of information on the parameters used for phototherapeutic resources is a persisting concern [16]. Therefore, clinical trials combined with technical/experimental studies may provide these data and consequently promote standardization of therapeutic protocols in the future.

Another important parameter verified was the heat generated by the device. This is important because the light emitter is used for application in the vaginal mucosal region. The literature defines LED treatment as a cold therapy [12, 13]. Our results showed that there was an increase in temperature of approximately 7 °C when the equipment was tested in the laboratory. Henderson et al. found a temperature increase of around 3 ± 0.3 °C when using laser light associated with photosensitizers in rat tumor cells, with important physiological effects such as increased congestion and vascular hemorrhage [17].

This study demonstrated the use of ultraviolet A/blue LED with a wavelength of 401 ± 5 nm in the treatment of a patient with a clinical and laboratory diagnosis of VVC. The

Fig. 5 Length of the speculum was used the application of the LED

Fig. 6 Maximum aperture of the speculum was used in the application of the LED

treatment led to the resolution of the clinical manifestations and the absence of fungal growth after the application of the technique, as confirmed by microbiological examination. It is believed that phototherapy, at the wavelength used in this study, is capable of inactivating microorganisms through the mechanism of pathogenic cellular death; that is, the blue light interacts with the porphyrins present in *Candida*, resulting in the production of reactive oxygen species and the consequent death of microbial cells [9, 10, 18, 19]. In addition, another explanation for the negative microbiological examination after the application of the technique may be related to the increase of vaginal pH, as *Candida* proliferates in an acidic environment [6].

A similar result was found in the study by Robatto et al., who used ultraviolet A/blue LED light in the 401 ± 5 nm range for the treatment of a patient with recurrent VVC. Three sessions were performed, which reduced the fungal load at the end of treatment. Three months after the conclusion of the treatment, the absence of fungal infection was noted [20]. They also observed resolution of the clinical manifestations (pruritus, burning, dysuria, dyspareunia, and edema) of the patient after treatment.

In the present study, a single session was conducted, based on the study of Ganz et al., who found positive results after the application of 405 ± 2 nm blue light to the gastric mucosa for the treatment of symptomatic patients with *Helicobacter pylori* infection. In the gastric mucosa study, at least 90% of the bacteria were eliminated in seven of the nine patients treated. These authors confirmed the results of their study by exposing endogenous porphyrins to blue light, which generated reactive singlet oxygen and caused microbial cell death through the rupture of organelles and chromosomal genetic material [21].

In our study, it was observed that the symptoms (pruritus and burning) and signs (lumpiness and vulvar erythema)

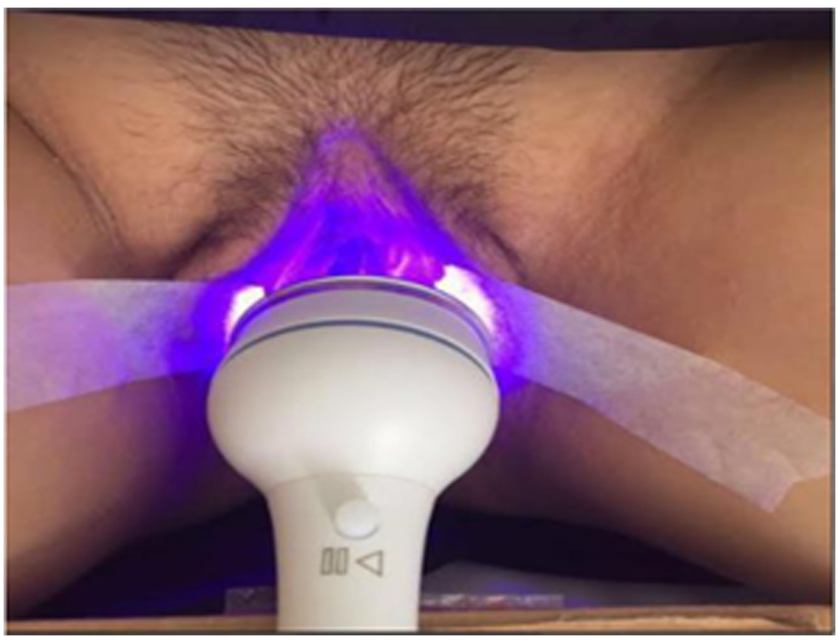

Fig. 7 Application of LED 401 ± 5 nm in vulva region

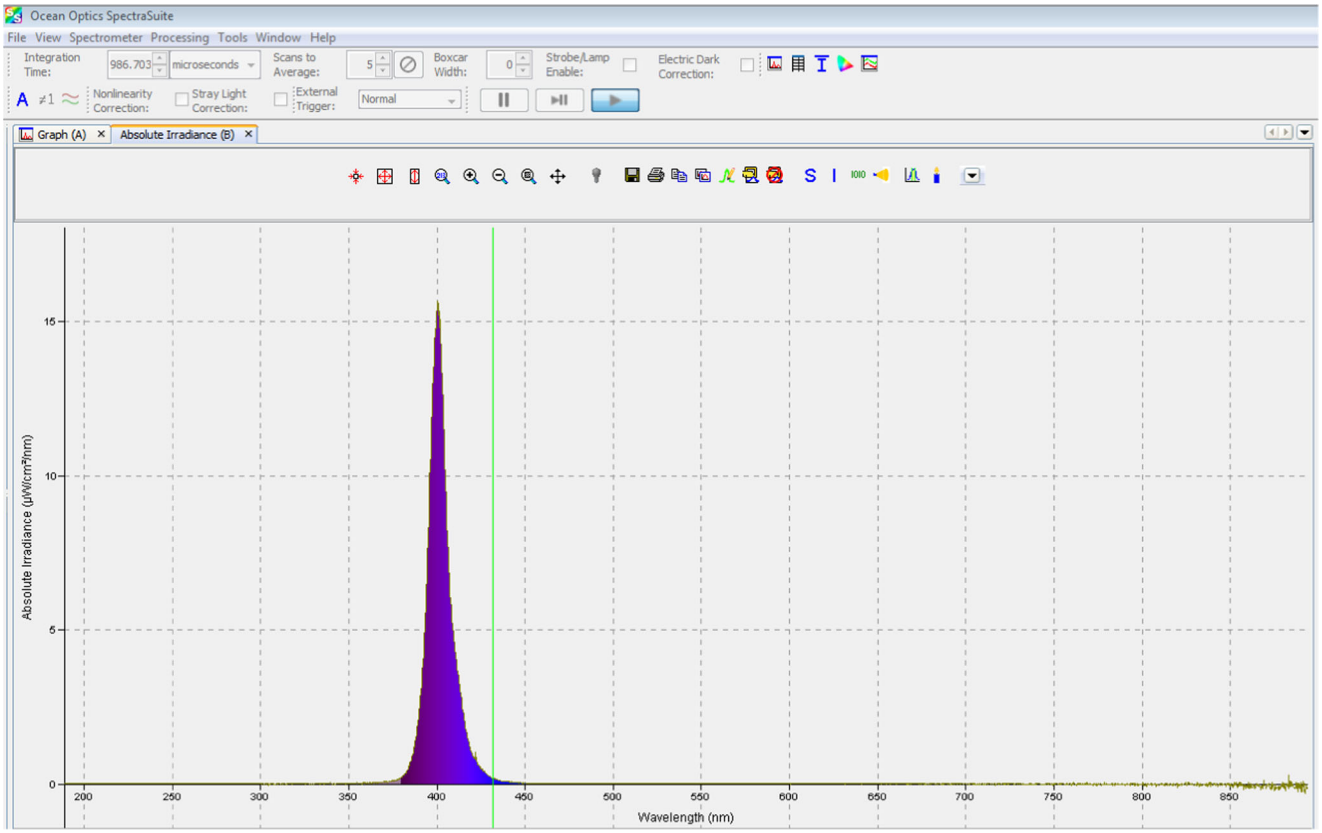

Fig. 8 Luminous spectrum measured at a distance of 5 cm from the source at maximum power

disappeared after a single application of blue light. This result can be explained by the absence of fungal elements in the patient's vaginal region after the treatment, owing to photoexcitation of the porphyrins present in fungi, leading to the production of reactive oxygen species and consequent cell death [11]. Gold et al. observed clinical improvement of acne and bacterial lesions after four sessions of blue LED treatment at 414 nm, with a reduction of erythema and local inflammatory lesions. They explained their finding as a result of the presence of porphyrins in *Propionibacterium acnes*, which, by absorbing blue light, generated singlet oxygen and subsequently caused bacterial cell death [22].

In our study, a reduction of inflammation was observed after treatment. This result can be explained by the absence of *Candida* after treatment, with consequent reduction of the inflammatory signs.

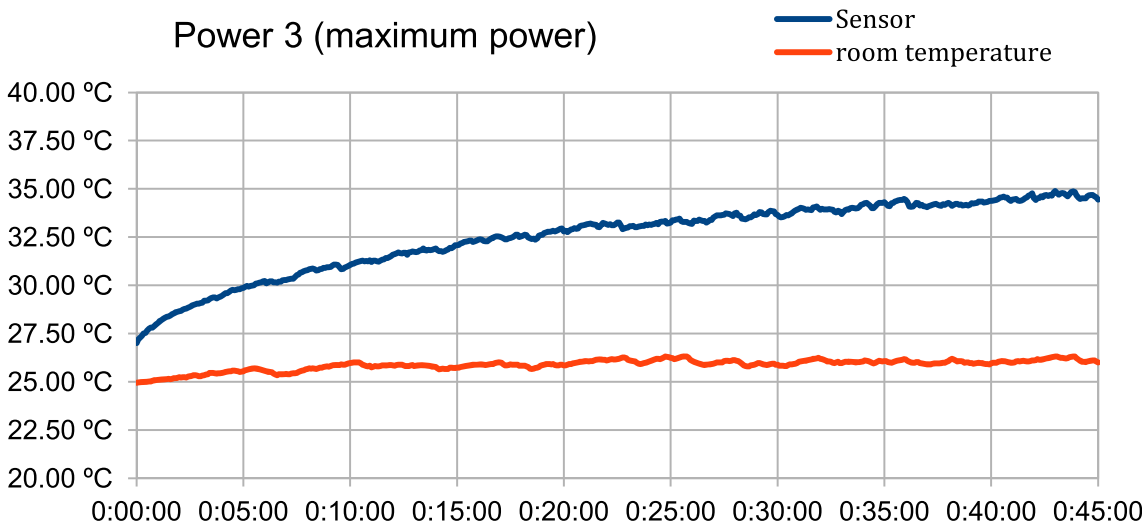

Fig. 9 Graphs generated by the different temperatures collected in a total time of 45 min, with *x*-axis in time and axis *y* in temperature

The patient reported no adverse effects during the session or after the end of treatment. This result may be explained by the fact that the therapeutic technique with LED has a superficial action [12, 13].

In this study, the light was applied for 60 min, divided into two applications, with 30 min each. However, we are aware of the need to reduce the treatment time, and we are planning future studies to test the optimal duration for this method. Because an LED device for simultaneous application of light onto the vulva and vagina has already been developed, the treatment period can be reduced to 30 min of light application.

The results of this treatment are preliminary and represent our experience in a single patient. Therefore, controlled clinical trials with a larger number of patients should be performed to evaluate the safety and efficacy of the therapy.

Conclusion

In the technical/experimental safety test, the light-emitting device showed emission in the ultraviolet A/blue (401 ± 5 nm) regions, and visible in the violet and blue ranges, with a temperature increase of approximately 7 °C.

Irradiation with ultraviolet A/blue LED may be an alternative treatment for VVC, as it led to the resolution of the clinical and microbiological manifestations in our patient, with no adverse effects. Randomized clinical trials with a larger sample size are warranted to evaluate the efficacy and safety of this innovative technique.

Acknowledgments This work would not have been possible without the intellectual discussions about the research of the PhD Physiotherapist Caroline Constante and Engineer Décio Minalle (DGM Eletronica).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the declaration of Helsinki of 1964 and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from the patient included in the study.

References

1. Chew SY, Than LT (2016) Vulvovaginal candidosis: contemporary challenges and the future of prophylactic and therapeutic approaches. Mycoses. 59(5):262–273. https://doi.org/10.1111/myc.12455
2. Liu MB, Xu SR, He Y, Deng GH, Sheng HF, Huang XM et al (2013) Diverse vaginal microbiomes in reproductive-age women with vulvovaginal candidiasis. PLoS One 8(11):e79812. https://doi.org/10.1371/journal.pone.0079812
3. Fidel PL Jr, Barousse M, Espinosa T, Ficarra M, Sturtevant J, Martin DH et al (2004) An intravaginal live *Candida* challenge in humans leads to new hypotheses for the immunopathogenesis of vulvovaginal candidiasis. Infect Immun 72(5):2939–2946. https://doi.org/10.1128/IAI.72.5.2939-2946.2004
4. Apalata T, Longo-Mbenza B, Sturm A, Carr W, Moodley P (2014) Factors associated with symptomatic vulvovaginal candidiasis: a study among women attending a primary healthcare clinic in Kwazulu-Natal, South Africa. Ann Med Health Sci Res 4(3):410–416. https://doi.org/10.4103/2141-9248.133470
5. Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P (2012) Epidemiologic features of vulvovaginal candidiasis among reproductive-age women in India. Infect Dis Obstet Gynecol 2012: 859071. https://doi.org/10.1155/2012/859071
6. Mårdh PA, Rodrigues AG, Genç M, Novikova N, Martinez-de-Oliveira J, Guaschino S (2002) Facts and myths on recurrent vulvovaginal candidosis–a review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. Int J STD AIDS 13(8):522–539. https://doi.org/10.1258/095646202760159639
7. Dovnik A, Golle A, Novak D, Arko D, Takač I (2015) Treatment of vulvovaginal candidiasis: a review of the literature. Acta Dermatovenerol Alp Pannonica Adriat 24(1):5–7. https://doi.org/10.15570/actaapa.2015.2
8. Xie HY, Feng D, Wei DM, Mei L, Chen H, Wang X et al (2017) Probiotics for vulvovaginal candidiasis in non-pregnant women. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD010496.pub2.
9. Murdock LE, Mckenzie K, Maclean M, Macgregor SJ, Anderson JG (2013) Lethal effects of high-intensity violet 405-nm light on *Saccharomyces cerevisiae*, *Candida albicans* and on dormant and germinating spores of *Aspergillus niger*. Fungal Biol 117(7–8): 519–527. https://doi.org/10.1016/j.funbio.2013.05.004
10. Imada K, Tanaka S, Ibaraki Y, Yoshimura K, Ito S (2014) Antifungal effect of 405-nm light on *Botrytis cinerea*. Lett Appl Microbiol 59(6):670–676. https://doi.org/10.1111/lam.12330
11. Guffey JS, Wilborn J (2006) In vitro bactericidal effects of 405-nm and 470-nm blue light. Photomed Laser Surg 24(6):684–688. https://doi.org/10.1089/pho.2006.24.684
12. Ablon G (2018) Phototherapy with light emitting diodes: treating a broad range of medical and aesthetic conditions in dermatology. J Clin Aesthet Dermatol 11(2):21–27
13. Barolet D (2008) Light-emitting diodes (LEDs) in dermatology. Semin Cutan Med Surg 27(4):227–238. https://doi.org/10.1016/j.sder.2008.08.003
14. Adamskaya N, Dungel P, Mittermayr R, Hartinger J, Feichtinger G, Wassermann K et al (2011) Light therapy by blue LED improves wound healing in an excision model in rats. Injury. 42(9):917–921. https://doi.org/10.1016/j.injury.2010.03.023
15. Watson C, Calabretto H (2007) Comprehensive review of conventional and non-conventional methods of management of recurrent vulvovaginal candidiasis. Aust N Z J Obstet Gynaecol 47(4):262–272. https://doi.org/10.1111/j.1479-828X.2007.00736.x
16. Jenkins PA, Carroll JD (2011) How to report low-level laser therapy (LLLT)/photomedicine dose and beam parameters in clinical and laboratory studies. Photomed Laser Surg 29(12):785–787. https://doi.org/10.1089/pho.2011.9895
17. Henderson BW, Waldow SM, Potter WR, Dougherty TJ (1985) Interaction of photodynamic therapy and hyperthermia: tumor response and cell survival studies after treatment of mice in vivo. Cancer Res 45(12 Pt 1):6071–6077
18. Maclean M, Mckenzie K, Anderson JG, Gettinby G, Macgregor SJ (2014) 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infections control. J Hosp Infect 88(1):1–11. https://doi.org/10.1016/j.jhin.2014.06.004
19. Inamura T, Tatehara S, Takebe Y, Tokuyama R, Ohshima T, Maeda N et al (2014) Antibacterial and antifungal effect of 405 nm monochromatic laser on endodontopathogenic. Int J Photoenergy:1–7. https://doi.org/10.1155/2014/387215
20. Robatto M, Pavie MC, Tozetto S, Brito MB, Lordêlo P (2017) Blue light emitting diode in treatment of recurring vulvovaginal candidiasis: a case report. Braz J Med Hum Health 5(4):162–168. https://doi.org/10.17267/2317-3386
21. Ganz RA, Viveiros J, Ahmad A, Ahmadi A, Khalil A, Tolkoff J et al (2005) *Helicobacter pylori* in patients can be killed by visible light. Lasers Surg Med 36(4):260–265. https://doi.org/10.1002/lsm.20161
22. Gold MH, Sensing W, Biron JA (2011) Clinical efficacy of home-use blue-light therapy for mild-to moderate acne. J Cosmet Laser Ther 13(6):308–314. https://doi.org/10.3109/14764172.2011.630081