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Clinical and morphopathological assay in vulvovaginal candidiasis

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Abstract

Candida vulvovaginitis is characterized by the appearance of inflammatory changes in the vaginal and vulvar epithelium secondary to infection with *Candida* species. The purpose of this study was to analyze and compare the clinical, microbiological, and histopathological aspects of pregnant and non-pregnant patients, symptomatic or asymptomatic in the case of candida vaginitis and to correlate the microscopic aspects with the symptoms before applying the local treatment with Nystatin. The study presents a retrospective analysis of the management of vaginitis in 166 pregnant or non-pregnant patients during 2021–2022. We observed the structure of the Malpighian squamous epithelium without keratinization present on the vaginal mucosa and the structure of the subepithelial connective tissue, which shows increased numerical values of inflammatory and vascular cellularity in the case of candida vaginitis symptomatic compared to asymptomatic ones. We noticed also in the microscopic study that in cases of asymptomatic patients before treatment, the number of inflammatory cells and blood vessels situated immediately under the epithelium was significantly lower compared to their number in symptomatic patients before treatment. Analyzing the results obtained after the administration of the treatment proposed by us, we can say that local Nystatin treatment is beneficial and safe for pregnant and non-pregnant patients and is a good alternative for patients with recurrent vulvovaginal candidiasis.

Keywords: vulvovaginal candidiasis, inflammation, vaginal mucosa, local treatment.

Introduction

Candida vulvovaginitis is characterized by the appearance of inflammatory changes in the vaginal and vulvar epithelium secondary to infection with *Candida* species [1, 2]. *Candida albicans* species (very common, in over 90% of cases) or *non-albicans Candida* (NAC) species, such as *C. glabrata*, *C. tropicalis*, *C. krusei*, and *C. parapsilosis* (10% of cases) are part of the normal vaginal flora in many women and are a type of fungi that present themselves like eukaryotic organisms found in the form of yeasts, molds or dimorphic fungi [2–4]. These microorganisms can become pathogenic especially when favorable conditions occur [3, 5]. The risk factors incriminated in favoring the appearance of this type of infection are represented by the administration of certain drugs (hormonal treatments, oral contraceptives, antibiotics, corticotherapy), physiological or pathological conditions characterized by hyperestrogenism (pregnancy, obesity), various conditions such as diabetes, conditions of immunosuppression [oncological patients undergoing chemotherapy, patients diagnosed with human immunodeficiency virus (HIV) infection or patients undergoing a transplant], unprotected sexual contact, poor hygiene conditions, etc. [1, 3, 4, 6].

Vaginal *Candida* infection occurs when these species of fungi superficially penetrate the mucosa of the vagina, thus causing a local inflammatory response. The dominant inflammatory cells are usually polymorphonuclear cells and macrophages [4, 7, 8].

According to the studies published so far, the frequency of this infection is quite high, being responsible for approximately one third of all cases of vulvovaginitis in women of reproductive age, and 75% of women report having had candida vulvovaginitis at some point in life [5, 9]. The highest incidence of candida vulvovaginitis is in sexually active women of reproductive age. However, there is no clear evidence that the infection is sexually transmitted [6, 7, 10].

Recurrent vulvovaginitis is a pathological condition of great clinical and social importance and is defined by the

occurrence of four or more episodes of candida vulvovaginitis proven by vaginal secretion culture examination. These patients have been shown to have predisposing genetic factors that make them susceptible to recurrent fungal infections and may also have a hypersensitivity to *Candida*. About 8% of women suffer from recurrent infection [5, 6, 8, 11].

The incriminated symptomatology is quite varied, from completely asymptomatic cases or the patients may complain of modified vaginal secretion, rough white, itching, burning sensation, and dyspareunia. During the local gynecological examination, in the case of patients with acute infection, dense, white vaginal secretions with rough deposits and/or vulvar and vaginal edema and erythema, local excoriations can be observed [9, 12].

The certainty diagnosis of this condition is made based on the culture examination, the treatment being based on the antifungigram related to this test. It is imperative to exclude another etiology of vaginal infection (especially bacterial vaginosis, gonococcal disease, and *Chlamydia*, *Ureaplasma*, *Mycoplasma*), already knowing that this infection can be easily confused or can be concurrent with other vaginal infections [10, 13].

The importance of this pathology resides in the fact that it can affect the lifestyle, as normal daily activities can be modified, with negative consequences from an emotional, social, and financial point of view (the diagnostic and therapeutic process can be quite expensive and long-lasting) [11, 14, 15].

Aim

The purpose of this study was to analyze and compare the clinical, microbiological, and histopathological aspects of pregnant and non-pregnant patients, symptomatic or asymptomatic in the case of candida vaginitis and to correlate the microscopic aspects with the symptoms before applying the local treatment with Nystatin.

Patients, Materials and Methods

This study presents a retrospective analysis of the management of vaginitis in 166 pregnant or non-pregnant patients, treated in the Clinic of Obstetrics–Gynecology, Filantropia Municipal Hospital, Craiova, Romania, during 2021–2022.

All investigated patients verbally and in writing consented and signed their agreement for participation in this study.

The patients were clinically examined, some were investigated before hospitalization, others were not investigated. The two groups of patients were subdivided percentageally into investigated and non-investigated patients, symptomatic or asymptomatic, from rural or urban areas, being treated or not, depending on the associated pathology.

Also in this study, in 20 (12.05%) patients (6.02% symptomatic before the application of the treatment and 6.02% asymptomatic) who gave birth vaginally and presented vaginal or perineal ruptures, tissue was collected for the microscopic study. The tissues were fixed in 10% neutral buffered formalin solution and processed for paraffin embedding. The obtained blocks were sectioned using an HM350 microtome equipped with a section transfer in a water bath system (STS microM) at a thickness of 4 μm. The obtained sections were stained by the classical staining techniques: Hematoxylin–Eosin (HE) and Goldner–Szekely trichrome (GST), but also by special immunohistochemical (IHC) staining techniques. The slides obtained were deparaffinized in xylene, rehydrated through alcohol baths of decreasing concentration of 100%, 90%, 70% (five minutes each) and then the rehydration was completed with distilled water (dH_2O) for 15 minutes. The special IHC techniques required antigenic exposure, performed with Citrate solution pH 6 and with Ethylenediaminetetraacetic acid (EDTA) pH 9. Also required: inactivation of endogenous peroxidase by applying 3% hydrogen peroxide (H_2O_2) solution, for 30 minutes, blocking non-specific sites with the help of skimmed milk solution for 30 minutes. After these first steps, the primary antibody was applied (Table 1) and the slides were left at 4°C (for 18 hours). On the second day, after removing the primary antibody and washing the slides with phosphate-buffered saline (PBS) solution, the secondary mouse/rabbit immunoglobulin G (IgG) antibody, VC002-025, R&D Systems, VisUCyte Horseradish peroxidase (HRP) Polymer was applied (one hour). The slides were developed with the help of 3,3'-Diaminobenzidine (DAB) (Dako), the specific elements being immunolabeled in brown, and the nuclei were highlighted with Hematoxylin solution. Afterwards, the slides were dehydrated with alcohol of increasing concentration 70%, 90%, 100% (five minutes each), clarified with xylene for 45 minutes and mounted with the coverslip and Canada balsam.

Table 1 – ***IHC panel of antibodies used by us***

Antibody	Manufacturer	Clone	Antigenic exposure	Secondary antibody	Dilution	Labeling
Anti-CD34	Dako	QBEnd/10	Citrate, pH 6	Monoclonal mouse anti-human CD34 Class II	1:50	Neoformed blood vessels
Anti-Ki67	Dako	MIB-1	EDTA, pH 9	Monoclonal mouse anti-human Ki67	1:50	Cells in division in the G1, S, G2 and M phase
Anti-CD68	Dako	KP1	Citrate, pH 6	Monoclonal mouse anti-human CD68	1:100	Macrophages
Anti-tryptase	Dako	AA1	Citrate, pH 6	Monoclonal mouse anti-human mast cell tryptase	1:500	Mast cells
Anti-CD3	Dako	–	Citrate, pH 6	Polyclonal rabbit anti-human CD3	1:50	T-lymphocytes
Anti-CD20	Dako	L26	Citrate, pH 6	Monoclonal mouse anti-human CD20cy	1:50	B-lymphocytes
Anti-CD79$^{\alpha}$	Dako	JCB117	EDTA, pH 9	Monoclonal mouse anti-human CD79$^{\alpha}$	1:50	Plasma cells

CD: Cluster of differentiation; EDTA: Ethylenediaminetetraacetic acid; IHC: Immunohistochemical; Ki67: Marker of proliferation.

The slides were analyzed microscopically and scanned using a Nikon Eclipse 44i microscope equipped with a Nikon DS-5Mc 5 megapixel cooled charged-coupled device (CCD) and controlled by the Image-Pro Plus AMS 7 (Media Cybernetics, USA) image analysis package. For each case, four pictures were taken of the vaginal mucosa area, with the 20× objective, using constant manual exposure and lighting settings. All inflammatory cells and blood vessels were counted manually using the Image-Pro Plus program and the obtained data were entered into a Microsoft Excel document. Numerical averages were performed for each individual case and later for each individual category. With the help of the same program, the standard deviations were determined, the graphs were created and the two-sample *t*-test assuming equal variances comparative test was applied.

Ethical consideration

Pregnant or non-pregnant women participating in the study provided written informed consent for the use of clinical data or tissue samples. Based on the Declaration of Helsinki, the Ethics Committee of the University of Medicine and Pharmacy of Craiova approved this study.

Results

The total of 166 patients was divided into two groups: group I – pregnant women (PW), which represented 52.41%; and group II – non-pregnant women (Non-PW), which represented 47.59% (Figure 1).

7.83% of the total patients were part of the PW group and were not investigated at admission (uPW), 44.58% of the total patients were part of the PW group and were investigated before admission (iPW). 3.61% of the total number of patients were part of the Non-PW group and were not investigated upon admission (uNon-PW), and 44.98% of the total number of patients were part of the same group and were investigated before admission (iNon-PW) (Figure 2).

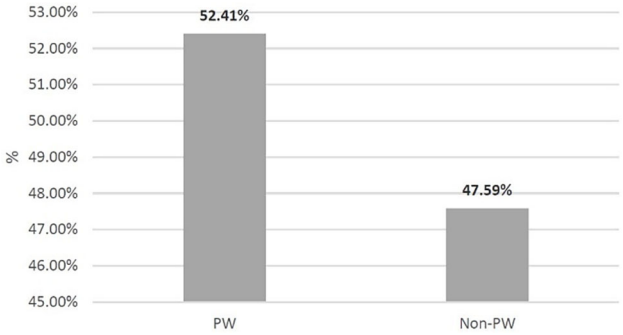

Figure 1 – ***The percentage of patients included in the study. Non-PW: Non-pregnant women included in the study; PW: Pregnant women included in the study.***

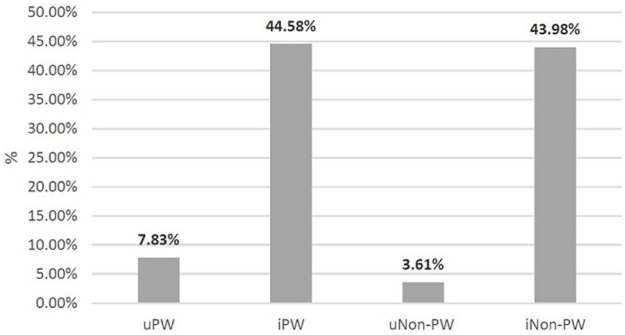

Figure 2 – ***The percentage of investigated/uninvestigated patients included in the study. iNon-PW: Investigated non-pregnant women; iPW: Investigated pregnant women; uNon-PW: Uninvestigated non-pregnant women; uPW: Uninvestigated pregnant women.***

The average age of patients in the uPW category was equal to 31.53 years (±8.36 years), of those in the iPW category was equal to 27.88 years (±5.27 years), of those in the uNon-PW category was equal to 35.50 years (±6.69 years) and of those in the iNon-PW category was equal to 31.26 years (±7.03 years). It is observed that the investigated patients from both PW/Non-PW categories presented a lower average age than the non-investigated ones (Figure 3).

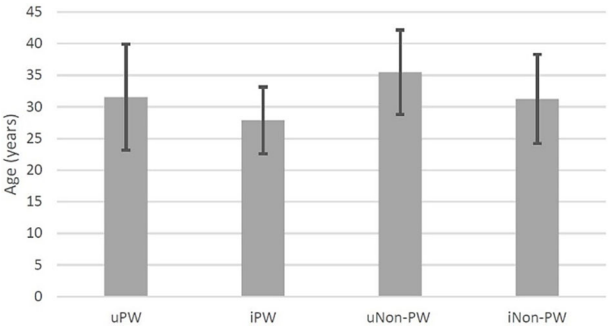

Figure 3 – ***The average age of the patients included in the study. iNon-PW: Investigated non-pregnant women; iPW: Investigated pregnant women; uNon-PW: Uninvestigated non-pregnant women; uPW: Uninvestigated pregnant women.***

From the 7.83% patients in the uPW group, 7.23% were from rural areas and 0.60% from urban areas; from the 44.58% patients in the iPW group, 15.66% were from rural areas and 28.92% from urban areas; all 3.61% of the patients belonging to the uNon-PW group were from the urban environment, and of the 44.98% of the patients belonging to the iNon-PW group, 5.42% were from the rural environment and 38.55% from the urban environment (Figure 4).

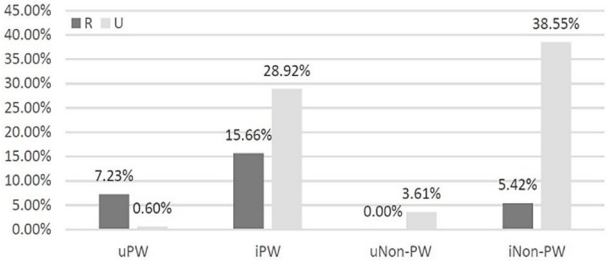

Figure 4 – ***The percentage of patients according to rural (R)/urban (U) environment. iNon-PW: Investigated non-pregnant women; iPW: Investigated pregnant women; uNon-PW: Uninvestigated non-pregnant women; uPW: Uninvestigated pregnant women.***

From the 7.83% pregnant women in the uPW group, 0.60% were asymptomatic and did not take local treatment with Nystatin and 7.23% presented associated symptoms (leucorrhea, itching, stinging). After the treatment, after 7 days only 1.20% of the patients still had symptoms and

after 14 days of treatment, no patient in the uPW group had any more symptoms (Figure 5).

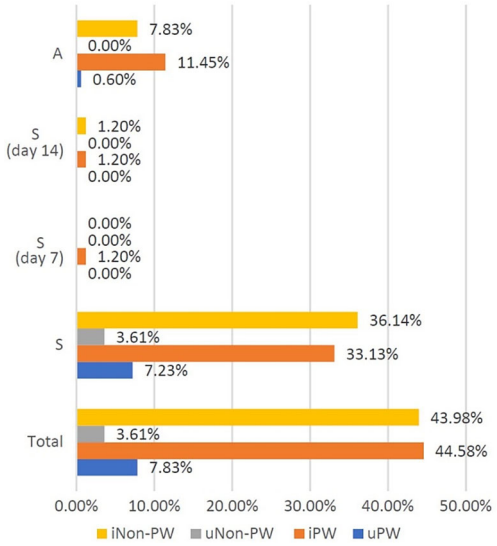

Figure 5 – ***The percentage of symptomatic patients treated at 7 and 14 days. A: Asymptomatic; iNon-PW: Investigated non-pregnant women; iPW: Investigated pregnant women; S: Symptomatic; uNon-PW: Uninvestigated non-pregnant women; uPW: Uninvestigated pregnant women.***

From the 44.58% pregnant women belonging to the iPW group, 33.13% were symptomatic and the culture was positive for *Candida* (4.22% of them having other infections associated with *Klebsiella* or *Escherichia coli*) and 11.45% were asymptomatic, although the culture was positive for *Candida* (of which 1.2% also had associated infection with *Gardnerella* and *Staphylococcus*). After the treatment, 1.2% of the patients continued to be symptomatic at 7 and 14 days, showing recurrent infection with *Candida* (Figure 5).

Regarding the group of Non-PW patients, all 3.61% uNon-PW presented symptoms (leucorrhea, itching, stinging), but following Nystatin treatment, at 7 and 14 days they were asymptomatic (Figure 5). Of the 44.98% iNon-PW patients, 36.14% were symptomatic and 7.83% asymptomatic. After the treatment, at 7 days no patient presented any more symptoms, but at 14 days, 1.2% of the symptomatic patients complained of symptoms again (Figure 5).

In the microscopic study, with the classical HE and GST staining, we observed the structure of the Malpighian squamous epithelium without keratinization present on the vaginal mucosa and the structure of the subepithelial connective tissue, which shows increased numerical values of inflammatory and vascular cellularity in the case of symptomatic candida vaginitis (Figures 6, A, C and D), compared to asymptomatic ones (Figure 6B).

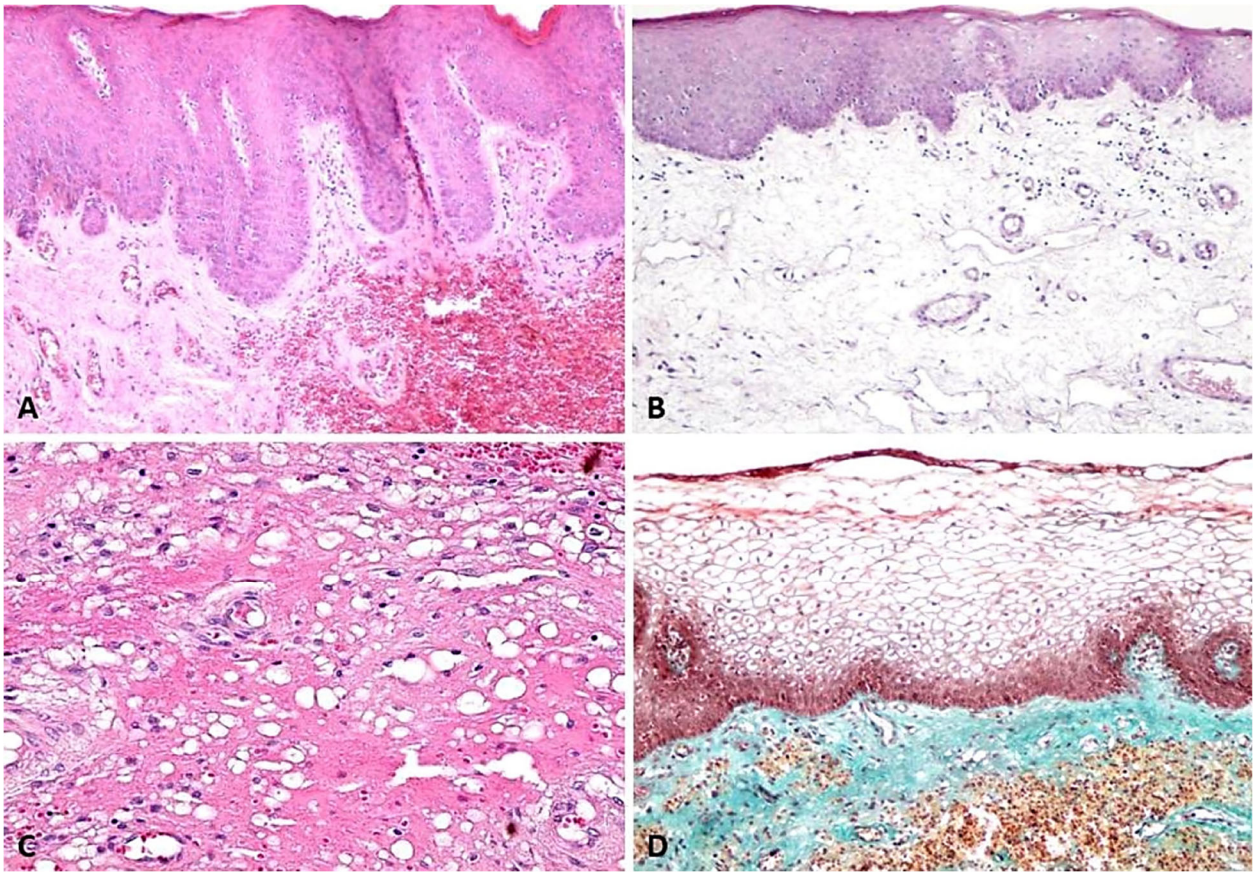

Figure 6 – ***The structure of the vaginal mucosa in symptomatic or asymptomatic patients with candida vaginitis: (A and C) Vaginal mucosa of a patient with symptomatic candida vaginitis before treatment; the extravasation of red blood cells in the subepithelial space and the increased density of inflammatory cellularity are observed; (B) Vaginal mucosa of a patient with asymptomatic candida vaginitis before treatment; the low number of inflammatory cells and blood vessels present subepithelially is observed; (D) Vaginal mucosa of a patient with symptomatic candida vaginitis before treatment; the extravasation of red blood cells in the subepithelial space and the increased density of inflammatory cellularity and green colored collagen fibers are observed. Classical HE staining: (A and C) ×200; (B) ×100. Classical GST staining: (D) ×100. GST: Goldner–Szekely trichrome; HE: Hematoxylin–Eosin.***

We noticed also in the microscopic study that in cases of asymptomatic patients before treatment, the number of inflammatory cells and blood vessels situated immediately under the epithelium was significantly lower compared to their number in symptomatic patients before treatment. The T-lymphocytes, highlighted with the anti-cluster of differentiation (CD) 3 antibody, varied from an average value of 273.25 cells/×200 to 376.75 cells/×200 in symptomatic patients, with a global average value equal to 305.53 cells/×200 (±43.28 cells/×200) (Figure 7A), and in asymptomatic patients they varied from a mean value of 33.5 cells/×200 to 62 cells/×200, with a global mean value equal to 43.28 cells/×200 (±8.71 cells/×200) (Figure 7B).

The B-lymphocytes, highlighted with the anti-CD20 antibody, varied from an average value of 38 cells/×200 to 55.25 cells/×200, with a global average value equal to 44.03 cells/×200 (±44.03 cells/×200) in symptomatic patients (Figure 7C), and in asymptomatic patients they varied from an average value of 9.50 cells/×200 to 18.25 cells/×200, with a global average value equal to 13.08 cells/×200 (±2.83 cells/×200) (Figure 7D).

Macrophages, highlighted with the anti-CD68 antibody, varied from an average value of 74 cells/×200 to 89.50 cells/×200, with a global average value equal to 79.68 cells/×200 (±79.68 cells/×200) in symptomatic patients before treatment (Figure 8A), and in asymptomatic patients it varied from 26.00 cells/×200 to 36.75 cells/×200, with an average global value equal to 29.85 cells/×200 (±3.34 cells/×200) (Figure 8B).

The plasma cells, highlighted with the anti-CD79$^{\alpha}$ antibody, varied from an average value of 11.75 cells/×200 to 17.25 cells/×200, with an overall average value equal to 15.70 cells/×200 (±15.70 cells/×200) in symptomatic patients (Figure 8C), and in asymptomatic patients it varied from 3.75 cells/×200 to 9.50 cells/×200, with a global average value equal to 7.03 cells/×200 (±7.03 cells/×200) (Figure 8D).

Mast cells, highlighted with the anti-tryptase antibody, varied from an average value of 18.50 cells/×200 to 25.50 cells/×200, with an overall average value of 21.65 cells/×200 (±2.40 cells/×200) in symptomatic patients (Figure 9A), and in asymptomatic patients it varied from an average value of 3.75 cells/×200 to 7 cells/×200, with an overall average value of 5.33 cells/×200 (±1.07 cells/×200) (Figure 9B).

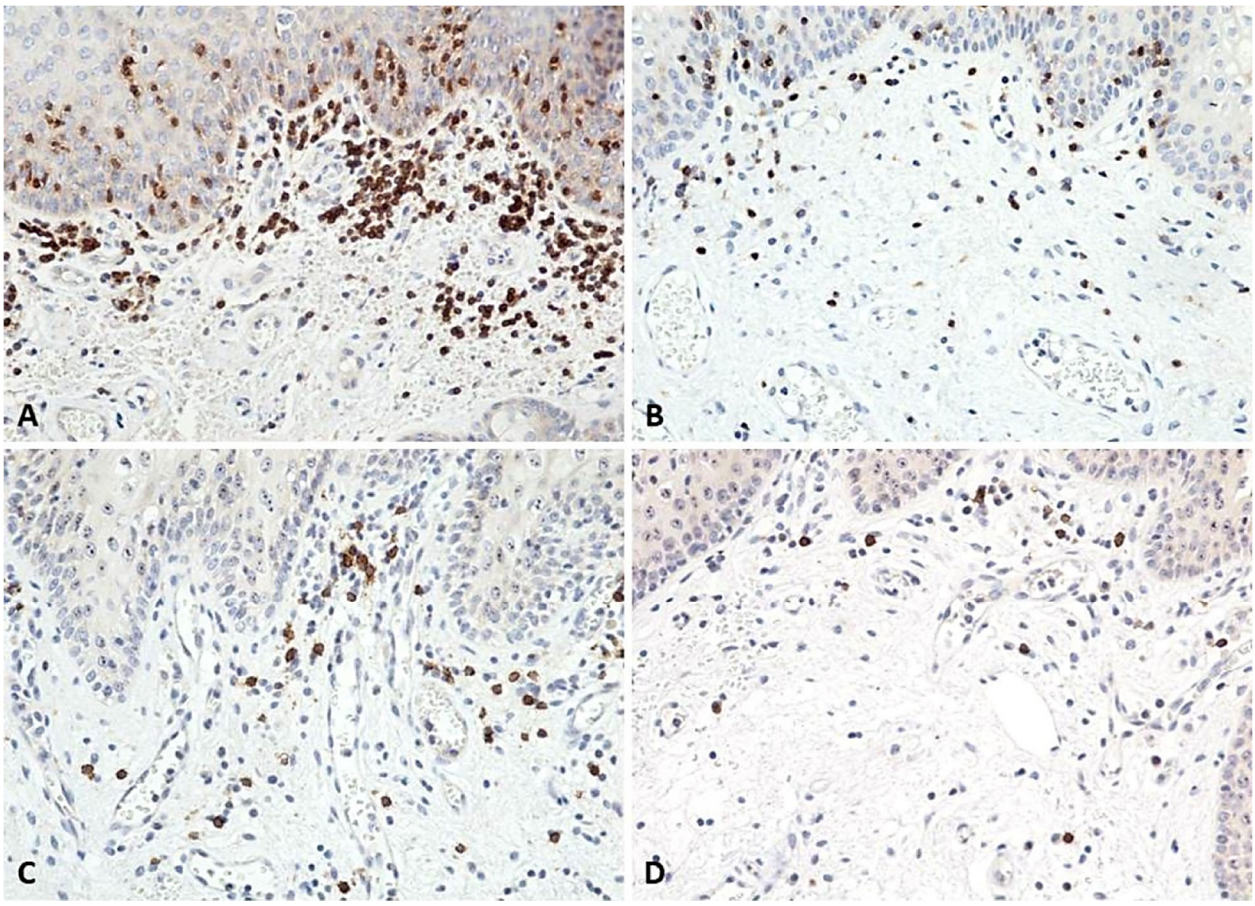

Figure 7 – ***The subepithelial appearance of the vaginal mucosa post-treatment in symptomatic or asymptomatic candida vaginitis: (A) Vaginal mucosa of a symptomatic patient before treatment, with subepithelial tissue showing an increased number of T-lymphocytes immunolabeled in brown with the anti-CD3 antibody; (B) Vaginal mucosa of an asymptomatic patient before treatment, with subepithelial tissue showing a low number of T-lymphocytes immunolabeled in brown with the anti-CD3 antibody; (C) Vaginal mucosa of a symptomatic patient before treatment, with subepithelial tissue showing an increased number of B-lymphocytes immunolabeled in brown with the anti-CD20 antibody; (D) Vaginal mucosa of an asymptomatic patient before treatment, with subepithelial tissue showing a low number of B-lymphocytes immunolabeled in brown with the anti-CD20 antibody, ×200. Anti-CD3 antibody immunolabeling: (A and B) ×200. Anti-CD20 antibody immunolabeling: (C and D) ×200. CD: Cluster of differentiation.***

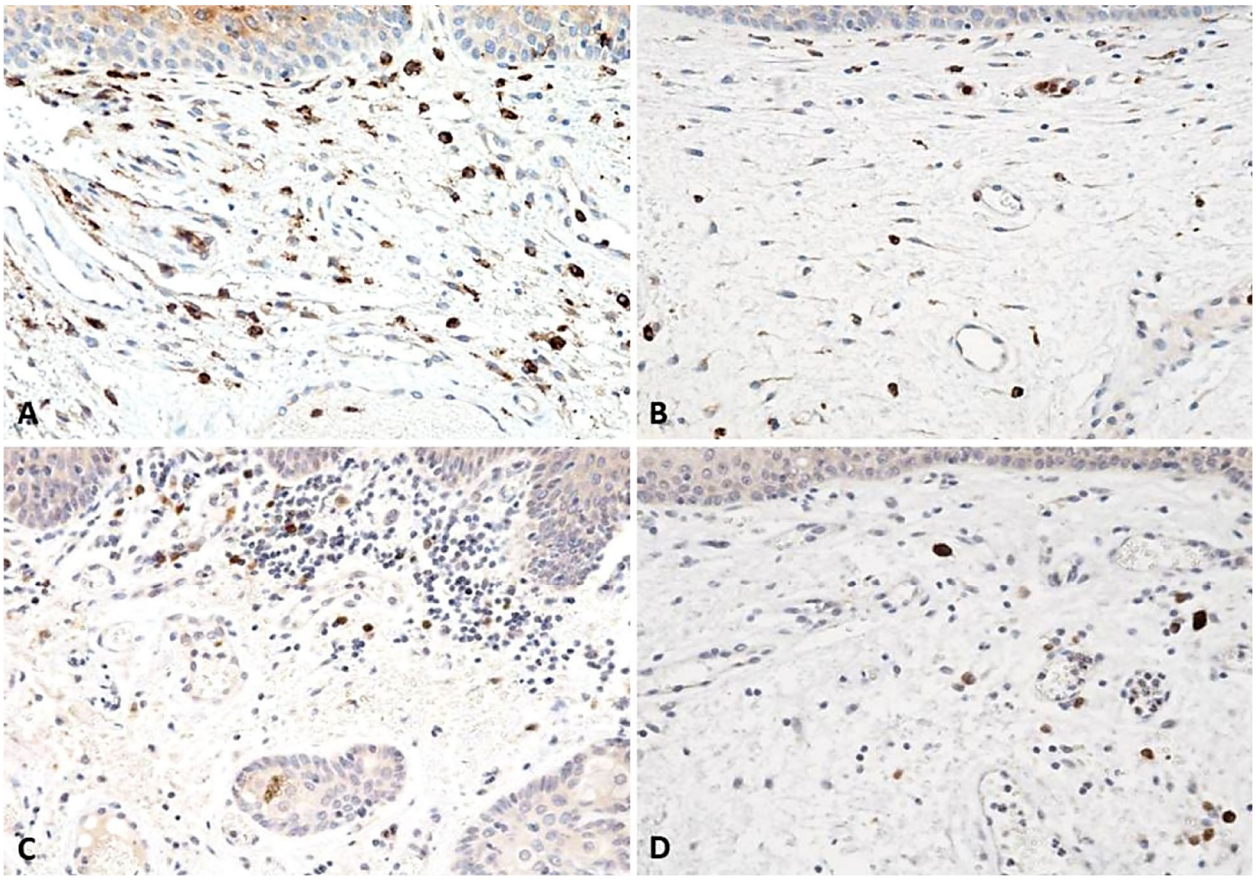

Figure 8 – ***The subepithelial appearance of the vaginal mucosa post-treatment in symptomatic or asymptomatic candida vaginitis: (A) Vaginal mucosa of a symptomatic patient before treatment, with subepithelial tissue showing an increased number of macrophages immunolabeled in brown with the anti-CD68 antibody; (B) Vaginal mucosa of an asymptomatic patient before treatment, with subepithelial tissue showing a low number of macrophages immunolabeled in brown with the anti-CD68 antibody; (C) Vaginal mucosa of a symptomatic patient before treatment, with subepithelial tissue showing an increased number of plasma cells immunolabeled in brown with the anti-CD79$^{\alpha}$ antibody; (D) Vaginal mucosa of an asymptomatic patient before treatment, with subepithelial tissue showing a low number of plasma cells immunolabeled in brown with the anti-CD79$^{\alpha}$ antibody, ×200. Anti-CD68 antibody immunolabeling: (A and B) ×200. Anti-CD79$^{\alpha}$ antibody immunolabeling: (C and D) ×200. CD: Cluster of differentiation.***

The capillary endothelium of the subepithelial blood vessels were highlighted using the anti-CD34 antibody, and their average numerical value varied from 19.50 blood vessels/×200 to 25.75 blood vessels/×200, with an average global value of 21.93 blood vessels/×200 (±2.03 blood vessels/×200) in symptomatic patients (Figure 9C), and in asymptomatic patients it varied from an average value of 10.75 blood vessels/×200 to 14 blood vessels/×200, with an average global value of 12.35 blood vessels/×200 (±1.33 blood vessels/×200) (Figure 9D).

Regarding the comparative study between asymptomatic patients before treatment and symptomatic ones, we noticed that there is a significant increase in inflammatory cells and subepithelial blood vessels in symptomatic patients, and by applying the two-sample *t*-test assuming equal variance between the categories, we observed that there are statistically significant differences between the two categories (Figure 10; Table 2).

The presence of *Candida* infection did not significantly influence the normal proliferative appearance of the basal layer of the Malpighian squamous epithelium without keratinization, in the intermediate and superficial layers dividing cells were not present in either symptomatic or asymptomatic patients. The proliferative basal layer was highlighted with anti-Ki67 antibody (Figure 11, A and B).

Discussions

Vulvovaginal candidiasis (VVC) is an infection localized to the mucosa of the vaginal and vulvar tissue and is mainly caused by *C. albicans*, an opportunistic fungal pathogen [1–3, 16, 17].

The methods of infection of the host by the *Candida* fungus can be multiple and are based either on its ability to evade the host's defense mechanisms or by adhering to the surface epithelium with the subsequent formation of a biofilm and invasion by the secretion of extracellular enzymes. As mentioned, adhesion of *Candida* to host surfaces is essential for primary colonization of human tissues, which is a prerequisite for infections [2, 7, 10–18]. Moreover, the production of hydrolytic enzymes induced by local infection can improve host organism adhesion, invasion and elimination of host immunological components and has a role in the acquisition of nutritional factors. Hydrolytic enzymes, such as secreted aspartyl proteinases (SAPs), phospholipases (PLs) and *Candida* species play a role in the pathogenesis of VVC [2, 8–19].

Numerous studies have reported that approximately 75% of women have experienced or been diagnosed with VVC [1–5, 20–25]. The maximum incidence of this infection is at reproductive age, an aspect also observed in our study,

the average age of the patients analyzed by us being between 27.88 years and 35.50 years. We were able to detect a discrepancy by comparing the groups of investigated and non-investigated patients and we noticed that the investigated patients from both PW/Non-PW categories had a lower average age than the non-investigated ones. This fact can be explained by the fact that recently young patients have had free access to medical information, which has led to an increase in the addressability of patients towards early diagnosis and treatment methods [20, 21].

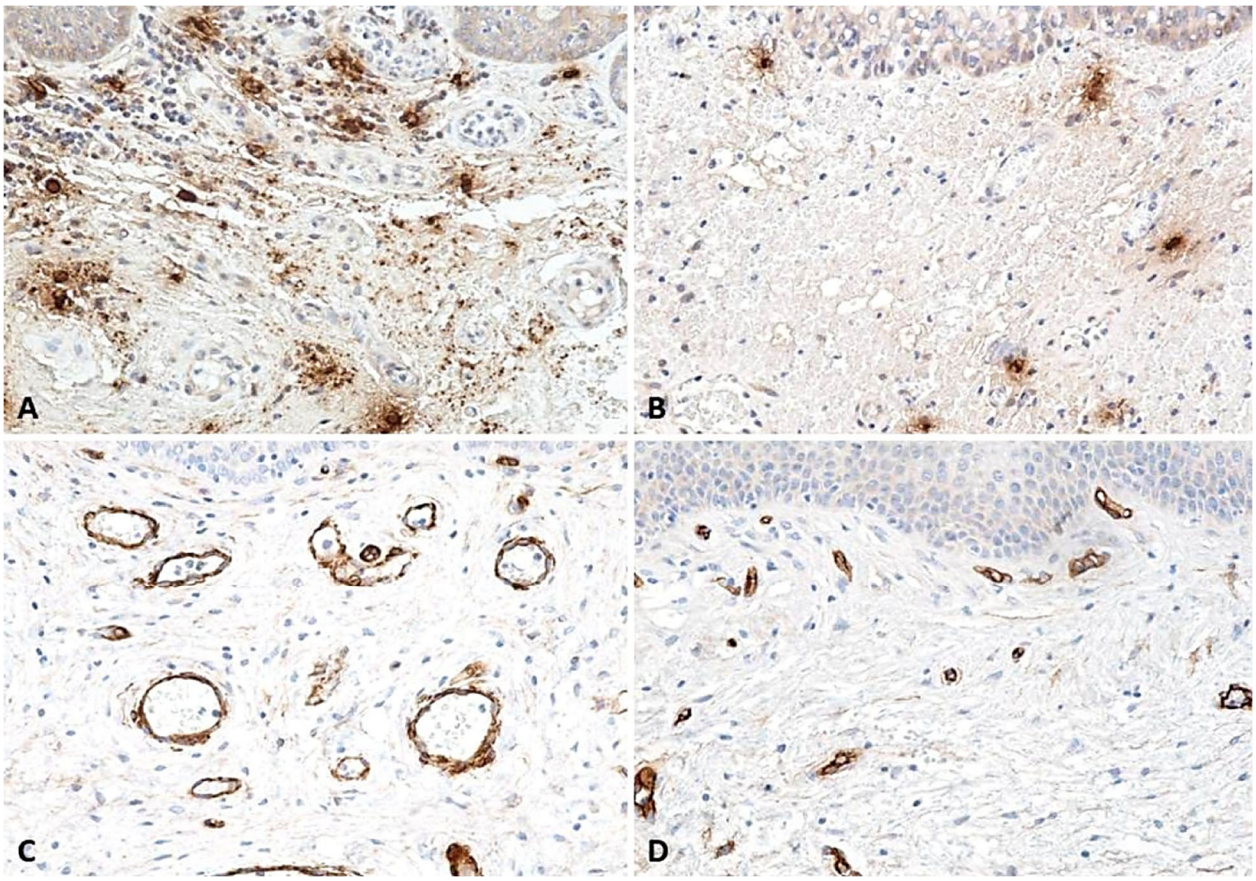

Figure 9 – ***The subepithelial appearance of the vaginal mucosa post-treatment in symptomatic or asymptomatic candida vaginitis: (A) Vaginal mucosa of a symptomatic patient before treatment, with subepithelial tissue showing an increased number of mastocytes immunolabeled in brown with the anti-tryptase antibody; (B) Vaginal mucosa of an asymptomatic patient before treatment, with subepithelial tissue showing a low number of mast cells immunolabeled in brown with the anti-tryptase antibody; (C) Vaginal mucosa of a symptomatic patient before treatment, with subepithelial tissue showing an increased number of blood vessels immunolabeled in brown with the anti-CD34 antibody; (D) Vaginal mucosa of an asymptomatic patient before treatment, with subepithelial tissue showing a low number of blood vessels immunolabeled in brown with the anti-CD34 antibody, ×200. Anti-tryptase antibody immunolabeling: (A and B) ×200. Anti-CD34 antibody immunolabeling: (C and D) ×200. CD: Cluster of differentiation.***

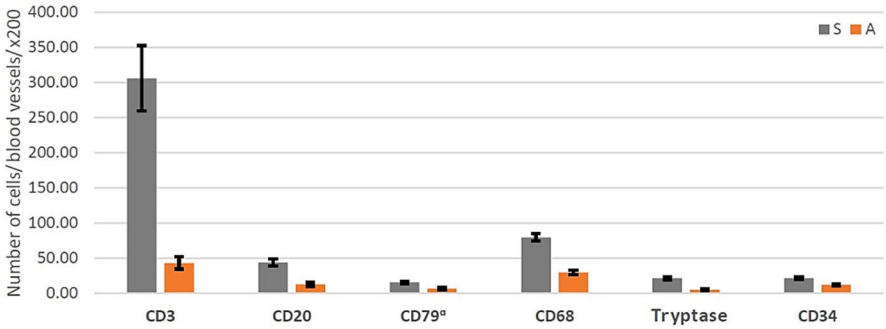

Figure 10 – ***The number of cells or blood vessels depending on the presence of symptoms before treatment. A: Asymptomatic; CD: Cluster of differentiation; S: Symptomatic.***

Table 2 – ***Comparison between the numerical values of inflammatory cells and blood vessels in asymptomatic and symptomatic patients before treatment***

	CD3	CD20	CD79ª	CD68	Tryptase	CD34
t Stat	17.56	16.65	11.84	25.52	19.62	12.44
P(*T*<=*t*) one-tail	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

CD: Cluster of differentiation.

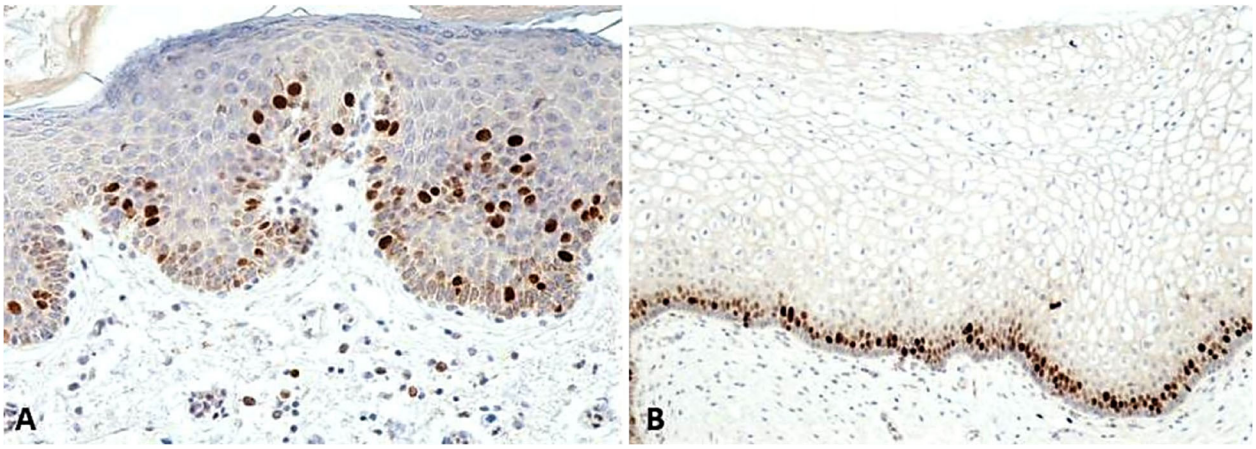

Figure 11 – ***The epithelial appearance of the vaginal mucosa post-treatment in symptomatic or asymptomatic candida vaginitis: (A) Vaginal mucosa of a symptomatic patient before treatment, with epithelial tissue showing the basal layer with dividing cells, immunolabeled in brown with the anti-Ki67 antibody; (B) Vaginal mucosa of an asymptomatic patient before treatment, with epithelial tissue showing the basal layer with dividing cells, immunolabeled in brown with the anti-Ki67 antibody. Anti-Ki67 antibody immunolabeling: (A) ×200; (B) ×100.***

Along with bacterial vaginosis, candidiasis is associated with symptoms such as pruritus, local irritation, dysuria, dyspareunia, and altered vaginal secretion, gritty/brownish white or local pain. This symptomatology can be supported and aggravated by the risk factors of this infection [2, 4, 25–30]. Poor hygienic conditions and lack of information and access to medical services can negatively influence the situation of patients diagnosed with this pathology. This observation was also highlighted in our study, where we concluded that the patients from the rural environment (a location characterized by the lack of specialized medical services) had a much larger share in the case of patients not investigated before admission (uPW, 7.23% were from rural areas and 0.60% from urban areas in the case of pregnant patients).

The symptomatology, as we mentioned, can be polymorphic. In the case of our patients included into the study, we observed completely asymptomatic cases but in a rather small proportion, 0.60% in the case of the uPW group, respectively 11.45% iPW and 7.83% iNon-PW. Conditions characterized by hyperestrogenism or immunodepression such as pregnancy can be a favorable factor for symptomatic *Candida* infection, a fact emphasized by the higher incidence of symptoms in the group of studied patients (from the proportion of 44.58% of pregnant women belonging to the iPW group, 33.13% presented symptoms). A similar study was published by Babić & Hukić who observed that symptomatic *Candida* infection is frequently encountered during pregnancy 40.9% (83/203) compared to the group of non-pregnant patients 23.8% (58/244) [13, 14].

The patients included in our study, after being diagnosed with *Candida* infection, benefited from Nystatin local treatment. We noticed that after a 7-day treatment with vaginal ovules with Nystatin, only 1.20% of the patients still had symptoms and after 14 days of treatment, no patient in the uPW group had anymore symptoms, and in the case of the patients in the iPW group, 1.2% of the patients had continued to be symptomatic at 7 and 14 days, showing recurrent infection with *Candida*. A similar result was also observed in the group of Non-PW patients, who after Nystatin treatment, at 7 and 14 days, were asymptomatic. A similar result was also published by Benedict *et al.*, who in their article pointed out that the patients' symptoms subsided after treatment with Nystatin [15]. Contrary to the results published by us, Phillips *et al.* claim that Clotrimazole, Miconazole, Terconazole and intravaginal Boric acid (BA) are much more effective in patients diagnosed with candida vaginitis. However, it is worth emphasizing that intravaginal Fluconazole and BA should be avoided during pregnancy [16].

Similar results were published by other authors, revealing the importance and major role of Nystatin treatment in candidiasis infection [29–31].

Also, in this study, we discovered the fact that the structure of the Malpighian squamous epithelium without keratinization present on the vaginal mucosa and the structure of the subepithelial connective tissue show increased numerical values of inflammatory cells. Watching the microscopic study, we noticed that in the asymptomatic patient before treatment, the number of inflammatory cells and blood vessels immediately under the epithelium were significantly lower compared to their number in symptomatic patients before treatment. A similar fact was also published by Balakrishnan *et al.* in their article, which emphasized the fact that T-lymphocytes can protect against *Candida* infections and the mediated decrease of T-lymphocytes found in immunosuppressed patients leads to an increased susceptibility to VVC in these patients. They also showed that immunity mediated by interleukin (IL)-17A and IL-17F can play a role in the host's defense mechanisms [2]. The increase in cell production on this level was also observed and reported by Steele & Fidel. They noticed that these high concentrations of IL-6 and IL-8 were produced by the epithelial cell layers in response to the inflammatory reaction [21, 27].

Yano *et al.* show in their article a fact that we have also observed, namely that *Candida* infection occurs predominantly in patients with T-cell immunodeficiency. At the same time, these authors also concluded that the mechanism by which a patient is resistant or susceptible in the case of vaginal infection with *Candida*, it is more closely related to dysfunctions of cellularity and local immunity, from the level of the vaginal mucosa, rather

than to systemic immunodepression [17, 32]. So, we can say that local topical treatment is more effective than systemic antimycotic treatment, the latter having side effects not to be neglected.

B-lymphocytes, macrophages, plasma cells, mast cells had a much higher concentration in symptomatic patients compared to asymptomatic patients. This fact can be a consequence of the infection, but it can also be a cause of the symptoms in these patients [18–20, 25]. The increase in the concentration of polymorphonuclear neutrophils (PMNs) at the level of the vaginal mucosa is a distinctive sign of an immunopathological inflammatory response that occurs in the case of acute infection with *C. albicans*. Yano *et al.* are trying to explain the role played by leukotrienes in candidiasis infection, highlighting and analyzing the leukotriene receptor antagonist (LTRA), namely LTB4 (Etalocib) or LTC4, LTD4 and LTE4 (Zafirlukast or Montelukast) and have demonstrated once again the fact that these receptors reduce the inflammation of epithelial tissues [18, 22, 23]. Also, according to Steele & Fidel, epithelial cells play active roles in the immune responses of the vaginal mucosa thus modulating the antimicrobial activity and the production of cytokines and chemokines in response to microorganisms [21].

The final conclusions are far from complete. Recent studies try to bring to the clinician's attention the importance of the vaginal microenvironment in the case of *Candida* infections. France *et al.* in their work emphasize the need to make progress in the integration of metagenomic, metatranscriptomic, metabolomic and immunological data, which could allow a detailed analysis of the microbiome–host relationship to obtain a specific and much more effective treatment [19, 26].

Conclusions

From this study, we observed the structure of the Malpighian squamous epithelium without keratinization present on the vaginal mucosa and the structure of the subepithelial connective tissue shows increased numerical values of inflammatory and vascular cellularity in the case of candida vaginitis symptomatic compared to asymptomatic ones. The presence of *Candida* infection did not significantly influence the normal proliferative appearance of the basal layer of the Malpighian squamous epithelium without keratinization, in the intermediate and superficial layers dividing cells were not present in either symptomatic or asymptomatic patients. Analyzing the results obtained after the administration of the treatment proposed by us, we can say that Nystatin treatment is beneficial and safe for pregnant and non-pregnant patients and is a good alternative for patients with recurrent VVC.

Conflict of interests

The authors declare that they have no conflict of interests.

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Authors' contribution

Maria Magdalena Manolea and Cosmin Vasile Obleagă equally contributed to this article.

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