Clinical, microbiological and therapeutic aspects of vulvovaginal candidiasis and recurrent vulvovaginal candidiasis: importance of regional surveys

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Abstract

Vulvovaginal candidiasis (VVC) has a varied aetiology. The yeasts involved in this disease have presented increased resistance to the azoles and poliens, the drugs of choice for treatment. In recurrent VVC, the therapeutic difficulties are even greater. The epidemiological relevance of aetiological diagnosis and of the antifungigram in clinical treatment is discussed.

Keywords: Candidiasis, Vulvovaginal. Recurrence. Diagnosis. Antifungal Agents.

1 Vulvovaginitis

The vulvovaginitis that is frequently diagnosed in gynaecological services refers to a variety of inflammatory disorders of the lower genital tract, which can be secondary to infection, irritation, allergy or systemic disease (ANGOTTI; LAMBERT; SOPER, 2007). It is the most common infection of the feminine genital tract, characterized by a triad of symptoms such as vulvovaginal pain, itching and burning (CAMARGO et al., 2008). In more than 90% of cases, the aetiology of vulvovaginitis includes yeasts, protozoans and bacteria (AKINBIVI et al., 2008; ANGOTTI; LAMBERT; SOPER, 2007; CHONG et al., 2007; EGAN; LIPSKY, 2000). The exact prevalence and cause of vulvovaginitis are uncertain due to the conditions of diagnosis and treatment, in spite of its asymptomatic nature and multifactorial causes (EGAN; LIPSKY, 2000).

Vulvovaginal candidiasis (VVC) is associated with different species of *Candida*, and in the United States and in other countries, is the second most common cause of acute vaginitis after unspecific bacterial vaginosis due to the GAM complex: *Gardnerella vaginalis,* anaerobes and *Mycoplasma* (ARECHAVALA et al., 2007; CHONG et al., 2007; EGAN; LIPSKY, 2000;). In these regions, bacterial vaginosis is generally the most common cause of vaginitis, being linked with 40–50% of cases in women of reproductive age. It has been found in 15–19% of outpatients in departments of gynaecology, in 10–30% of pregnant women and in 20–40% of patients in clinics for the treatment of sexually transmissible diseases (AKINBIYI; WATSON; WABOSO, 2008).

In Europe and several developed countries, VVC is the most common cause of vaginitis and it is expected that 75% of all women around the world will present, during their lifetime, with at least one episode of vulvovaginal candidiasis (ARECHAVALA et al., 2007; CHONG et al., 2003, 2007; EGAN; LIPSKY, 2000; REED et al., 2003). In Brazil, epidemiological data about the disease are scarce (ROSA; RUMEL, 2004). *Candida albicans* is the infectious agent that is recovered in 80–90% of patients and approximately 5–40% of women may develop recurrent episodes (CHONG et al., 2007; EGAN; LIPSKY, 2000; REED et al., 2003).

It is accepted that the trichomoniasis caused by *Trichomonas vaginalis* is the third most common cause of vaginitis, affecting 180 million women around the world, and accounting for 10–25% of vaginal infections. Its incidence, however, is declining in most developed countries (EGAN; LIPSKY, 2000; KLUFIO et al., 1995).

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In general, the vaginal discharge carries out cervical mucus and old cells that have lined the vagina. The amount of vaginal discharge varies with age, menstrual cycle, pregnancy and the use of oral contraceptives. The vaginal environment in healthy individuals is characterized by a dynamic relationship between *Lactobacillus acidophilus* and other members of the microbiota, estrogen, glycogen, vaginal pH and metabolic products of resident microorganisms and pathogens. *L. acidophilus* produces hydrogen peroxide, which is toxic for some microorganisms, and its fermentative activity contributes to the maintenance of vaginal pH between 3.8 and 4.2, contributing to the occurrence of yeasts of the *Candida* type (ATURNINO et al., 2005; EGAN; LIPSKY, 2000).

Vaginitis occurs when the vaginal microbiota is altered by the introduction of a pathogen or by alterations in the pH in the vaginal environment, permitting microbial proliferation (EGAN; LIPSKY, 2000; GROSS et al., 2007). *Candida* belongs to the human microbiota, being found especially in the skin and in mucous membranes. These yeasts may also be found in the female genital tract of approximately 20% of healthy women, with this rate increasing during pregnancy (ARECHAVALA et al., 2007).

In studies on the association of vaginal pH and the presence of vulvovaginitis, *Candida* sp. occurred more frequently at pH 4.0, *Trichomonas vaginalis* at pH 6.0, *Gardnerella vaginalis* at pH 5.0, and other bacteria between a pH of 4.0 to 6.0 (SATURNINO et al., 2005).

Overall, VVC is considered a public health issue that affects millions of women annually, creating great discomfort, interfering with sexual activity and affectionate relations and compromising the work performance of a significant number of the economically active population (HOLANDA et al., 2007).

1.1 Vulvovaginal candidiasis

VVC is one of the most frequent diagnoses in gynaecological practice and its incidence has increased drastically (HOLANDA et al., 2007; SHEEP et al., 2006). The predisposition for the development of these yeast infections is mediated by multiple factors, such as the level of health, the use of prosthetic products, the prior colonization of mucous membranes by microorganisms with potential virulence and their ability to adhere to the host tissues (EL-AZIZI; STARKS; KHARDORI, 2004). The disease is considered an opportunistic infection and a change from the asymptomatic to symptomatic condition indicates a transition of the saprophytic form of *Candida albicans* to the pathogenic form. Currently, the factors responsible for this transition and the mechanisms that result in the pathogenic behaviour of *C. albicans* are not clear (HOLANDA et al., 2007; SHINOBU et al., 2007).

Species of *Candida* are thought to be opportunistic pathogens, which depend on their own physiology and host predisposing factors to cause infection. Like other amphibiotic microorganisms belonging to the human microbiota, during homeostatic equilibrium, these yeasts remain in the mucous membranes as regular colonizers. However, under physiological imbalance in the host, *Candida* strains may reproduce excessively and express new features such as virulence factors (CAMARGO et al., 2008).

The pathogenic potential of *Candida* depends on several virulence factors, such as the ability to grow at 37 °C and to produce hyphae and pseudo-hyphae, representing a barrier for phagocytosis and allowing the fixation of the yeast onto the epithelium, the production of exoenzymes (phospholipases and proteinases) that help in the adherence of the yeasts to the mucous membrane and the mananas that promote the depression of the host immune system (CAMARGO et al., 2008; SHINOBU et al., 2007). Other virulence factors such as haemolytic activity and resistance to hydrogen peroxide have been considered, but their relevance in vaginal specimens should be studied in more detail (SHINOBU et al., 2007).

During the development of the infectious diseases caused by *Candida*, such as candidiasis, the process of adhesion of the yeast to the epithelial surface takes place, generally by the union of the conidium to a membrane receptor. Once joined, the conidia are not able to penetrate the vaginal epithelium. Their germination is necessary and pseudo-hyphae are produced (HOLANDA et al., 2007). The observation of filaments accompanying the blastoconidia via direct microscopic examination is an indication of the invasive capacity of *Candida*. In addition, the association of infectious disease symptoms with the production of the germ tube by the yeast has been demonstrated (SHINOBU et al., 2007; SUÁREZ; LANCHA, 2004). On the other hand, vaginal defenses against infection are determined by factors such as the microbiological balance, the integrity of the mucous membrane, the presence of the A and G immunoglobulins and the polymorphonuclear and monocyte populations (HOLANDA et al., 2007).

Given the endogenous origin of the disease, i.e. in an attempt to differentiate between asymptomatic and symptomatic colonization, the direct counting of microorganisms in clinical specimens was proposed, according to the criterion of Higashide

and collaborators, as described by Suárez and Lancha (2004). Up to 10 colony forming units from the primary isolation would be considered asymptomatic colonization, whereas more than 10 colony forming units would be indicative of infectious disease.

VVC may be associated with situations of weakness in the host, such as neoplasias and immunodeficiency. Several authors have suggested other predisposing factors such as antibiotics, immunosuppressive and antineoplasic drugs, oestrogen therapy, small traumas caused during the sexual act, the use of synthetic fabrics and tight fibres, and a diet of very acidic food, allergies (rhinitis and bronchial asthma) and the use of the intrauterine device (HOLANDA et al., 2007; ROSA; RUMEL, 2004). It is also accepted that any alteration in glucose levels, especially in situations of hyperglycemia, and any state in which the rates of vaginal glycogen become elevated, may unleash VVC. Thus pregnancy, the use of oral contraceptives and hormonal replacement therapy that result in episodes of hyperoestrogenism and thus high levels of glycogen, may favour VVC (SUÁREZ; LANCHA, 2004). The increase in the glycogen availability enhances the yeasts' growth and their ability for adhesion (ÁLVARES; SUIDZINSKI; COMALAVO, 2007). In addition, the presence of regular menstrual cycles has been identified as a risk factor for VVC, especially related to the oestradiol peak (HOLANDA et al., 2007; SUÁREZ; LANCHA, 2004).

With regards hygiene, it has also been suggested that hygiene carried out in the direction of the anus to the vagina, and residues of faeces in underwear, might be associated with vulvovaginal candidiasis (ÁLVARES; SUIDZINSKI; COMALAVO, 2007; HOLANDA et al., 2007; ROSA; RUMEL, 2004).

2 Clinical and microbiological considerations

VVC is clinically characterized by itching, burning, urination pain and a thick, white, odourless granulated vaginal discharge, and the vulva and vagina are frequently oedemic and hyperemic. The injuries may extend to the perineal, perianal and inguinal regions and, in typical cases, to the vaginal walls and the lip of the womb, with small yellow-white spots. The most common symptoms are itching, discharge, erythema and edema, which may intensify in the premenstrual period, when the acidity increases (ÁLVARES; SUIDZINSKI; COMALAVO, 2007; BOATTO et al., 2007).

Another studies describing the clinical characteristics of VVC, itching and vaginal discharge were also the most common symptoms, followed by erythema and edema, although *Candida* was only isolated from 52% of the patients evaluated, and there was no correlation between the yeasts isolated and the symptoms presented. Other studies have shown that patients with *C. albicans* complain of itching, leucorrhea and erythema more often than patients bearing other *Candida* species (BOATTO et al., 2007; HOLANDA et al., 2007). Itching has been observed to be the most important clinical symptom in patients with VVC, permitting differentiation from vaginitis of other aetiologies, in which it is less frequent. According to some authors, *Candida* species other than *C. albicans* may occur without symptoms in approximately 44% of cases (HOLANDA et al., 2007).

Although *C. albicans* is the most prevalent infectious agent associated with human VVC, other species, such as *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. guillermondi* and *C. krusei* are also highly associated (CAMARGO et al., 2008; CHONG et al., 2003; EGAN; LIPSKY, 2000; GROSS et. al., 2007; HOLANDA et al., 2007). Reports have shown the simultaneous participation of two or more species in a single disease episode (ARECHAVALA et al., 2007). Variation in the species by geographical location is related to the disease epidemiology (HOLANDA et al., 2007). VVC due to *C. albicans* is clinically indistinguishable from that due to other species that result in very similar symptomatology. However, it has been reported that *C. albicans* is more frequently associated with the symptoms than other species, which are more resistant to the usual therapies (SIMÕES, 2005).

3 Diagnostic aspects

The clinical diagnosis of VVC is suggested by the presence of the classic symptoms and must be confirmed by laboratory diagnosis, which involves microscopic examinations (blastoconidia structures are observed, associated or not with the pseudo-hyphae), isolation of the yeast in culture medium and metabolic tests to determine the species (CAMARGO et al., 2008). According to the same authors, the signs and symptoms were not associated with the isolation of *Candida* in culture. This suggests that there are no pathognomonic clinical manifestations of vulvovaginal candidiasis, and demonstrates the need for at least one microscopic exam and whenever possible culture in order to confirm the diagnosis. To summarize, self-diagnosis or diagnosis based only on the clinical experience of this disease, prior to an appropriate gynaecological examination, leads to many unnecessary treatments and lack of correct treatment of cases that do not present the classic symptomatology.

The most commonly used diagnostic methods for VVC are direct examination with KOH (20–40%) or coloured smears (Papanicolaou, Gram, Giemsa or Blue of Cresil) of the vaginal contents. The identification of the wrapped sort has not been employed in daily practice and is indicated in cases in which the symptomatology is highly suggestive and all previous tests were negative, or in a recurrence, to identify the species responsible (SHEEP et al., 2006).

Recently, there has been much interest in the use of Papanicolaou cytology to diagnose some of the cervico-vaginal infections associated with sexually transmitted pathogens. This reflects the fact that the technique is sensitive, cheap and highly reproducible. In the case of VVC, it allows the observation of yeasts, the pseudo-hyphae, and also cellular alterations indicating inflammation and/or infection. Cytology could be considered a morphological method with an efficiency very similar to the Gram stain for detecting vaginal yeasts, making it highly applicable in the routine laboratory (CARNEIRO et al., 2006). According to some authors, comparison of the accuracy of the clinical diagnosis with microbiological results in vaginal infections showed that only 43.2% of infections were confirmed by laboratory examinations, thus suggesting that clinical diagnosis is insufficient and that infection must be confirmed by microbiological tests (KARACA et al., 2005).

Analysis of the sensitivity and specificity of the clinical diagnosis showed that the prevalence of VVC was 19.3% and that the frequency of vulvovaginitis diagnosed by clinical examination was 17%, with a sensitivity of 38% and specificity of 88% (ROSA; RUMEL, 2004). These findings are consistent with those of the literature, where it has been noted that the symptoms do not differ between the three most common causes of vaginitis/vaginosis (bacterial, *C. albicans* and *T. vaginalis*) and that the presence of signs and symptoms has limited value, only diagnosing half (49%) of the patients that really have the infection. So, low accuracy has been demonstrated in predicting candidiasis based only on clinical findings, without laboratory confirmation.

The frequency of symptoms such as itching, irritation, leucorrhea and urinary pain is not known and such symptoms are unspecific. As a result, clinical and self-diagnosis without laboratory confirmation are questionable. The handling of vaginitis remains empirical, based on the consensus that vaginitis does not bring risk to life and that the empirical treatment is not damaging. The introduction of antifungal chemotherapy and self-medication was enthusiastically adopted by consumers and physicians, without worrying about the potential effects of the abusive and very often unsuitable use of medicines (ROSA; RUMEL, 2004).

4 Therapeutic considerations

In most cases, infection by *Candida* species can be treated by antimicrobial drugs, the antifungal azoles being the drugs of choice. Imidazolic and triazolic agents have been employed (fluconazole, miconazole, clotrimazole, itraconazole and ketoconazole), besides the poliens nistatine and amphotericin B (CARVALHO, 2003; GALLE; GIANINNI, 2004; FERRAZZA et al., 2005). However, some studies point to the resistance of *Candida* species to some of these compounds (ARECHAVALA et al., 2007; CHONG et al., 2007; FERRAZZA et al., 2005; GALLE; GIANINNI, 2004; SIMÕES, 2005; GROSS et. al., 2007).

Although the azoles represent a great advance in the treatment of systemic and local fungicidal infections, the inappropriate or overuse of these drugs in the treatment of yeast pathologies has contributed to the selection of resistant or less sensitive strains. Antifungal susceptibility patterns differ among *Candida* species, making it necessary to identify the aetiological agent before therapy (ROZKIEWICZ et al., 2005).

Given the lack of standardization in the techniques for evaluating susceptibility to antifungals, the literature recognizes the difficulty of comparing regional studies on the aetiology of VVC and its empirical treatment. Furthermore, there is a shortage of studies on the epidemiology and profiles of sensitivity to drugs of yeasts associated with VVC. This type of study is important, since many patients do not respond to initial therapy (FERRAZZA et al., 2005).

It has been suggested that the difficulties in the treatment of VVC are not only related to the susceptibility of the yeasts to the antifungal drugs, since there is a tendency for any of the *Candida* species other than *albicans* to require greater concentrations of antifungals for inhibition, as compared to *C. glabrata* treated with fluconazole (RIBEIRO et al., 2001). However, during pregnancy, the use of the topical imidazoles shows greater efficiency than nistatine. For therapy lasting seven days in uncomplicated VVC, instead of the short treatments that are commonly used in patients who are not pregnant, an alternative option is the use of imidazolic and triazolic agents, by oral or intravaginal route; however, the cost–benefit ratio and the wishes of the patient must be taken into consideration, since the efficiency appeared to be similar when the two forms of drug

administration were compared (YOUNG; JEWELL, 2001; NURBHAI et al., 2007).

With regards antimicrobial resistance, the use of fluconazole at low doses to prevent fungal infections in immunocompromised patients has caused the selection of yeasts of the microbiota, such as *C. grablata* and *C. krusei*, that are resistant to this antifungal agent (CARVALHO, 2003). It was also reported that most women who presented with recurrence showed a slightly higher occurrence of *C. glabrata*, which is less sensitive to the imidazolic drugs commonly used in the treatment of VVC and of recurrent vulvovaginal candidiasis (ÁLVARES et al., 2007).

5 Recurrent vulvovaginal candidiasis

As already mentioned, 5–40% of women with VVC have episodes of recurrence (CHONG et. al., 2007; EGAN; LIPSKY, 2000; REED et al., 2003). Recurrent vulvovaginal candidiasis (RVVC) is diagnosed when the patient presents up to four episodes of VVC in the period of one year (CHONG et. al., 2007; EGAN; LIPSKY, 2000), or up to three episodes in one year when not related to antibiotic therapy (CHONG et al., 2003). It is not known for certain whether the recurrence of VVC is secondary to predisposing/precipitant factors, sexual transmission, an intestinal reservoir or vaginal persistence (EGAN; LIPSKY, 2000).

Overall, it is accepted that two principal hypotheses are associated with the recurrence of candidiasis: a) reinfection through sexual transmission or from other reservoirs of the organism (digestive or urinary tract); b) reinfection due to the incomplete elimination of *Candida* species that have an increased virulence or are resistant to the antimicrobial drugs. A reduction in local cellular immunity may also be a risk factor for RVVC (CHONG et al., 2003).

In recent years, a significant increase in *Candida* species other than *albicans* that cause vaginitis has been observed, mainly in those cases connected with RVVC (CHONG et al., 2003). Allied to that, the occurrence of *Candida* strains that are resistant to antifungals has inspired some authors to propose not only microbiological diagnosis, but the additional performance of an antifungigram (ARECHAVALA et al., 2007; FERRAZZA et al., 2005;GALLE; GIANINNI, 2004).

In the Brazilian case, there are many questions about the necessity of laboratory diagnosis, especially for cases of RVVC, for which the value of mycological diagnosis is recognized by some authors, as well as the use of the antifungigram (FERRAZZA et al., 2005; GALLE; GIANINNI, 2004; RIBEIRO et al., 2001). Reports from gynaecological services have shown that at least half of the women who are referred as bearers of RVVC in fact have symptoms due to causes other than candidiasis (SIMÕES, 2005).

Several of the *Candida* species other than *albicans* that are commonly isolated are less sensitive to the azolic derivates, making the treatment of these infections more difficult. Although the antifungal susceptibility of the yeasts of the genus *Candida* is historically predictable, the susceptibility patterns of strains circulating nowadays are not easily predicted, especially given environmental selection and the cultural differences in the different regions. This is one of the reasons underlying the development of susceptibility tests for these drugs. Efforts to standardize these tests led to the establishment and regular update of antimicrobial testing manuals such as the document supplied by the Clinical and Laboratory Standards Institute, which is referred to in Brazilian microbiological practice (CLINICAL AND LABORATORY STANDARDS INSTITUTE, 2008). As an alternative to the CLSI recommendations to evaluate antimicrobial susceptibility, the E-test method, which is used to check susceptibility to the antifungals *in vitro*, is clinically validated to predict the susceptibility patterns of yeasts in a more simple way and can easily be incorporated into the clinical microbiology routine (CROCCO et al., 2004).

6 Final considerations

Despite the CLSI recommendations, there is still a lack of standardization in the techniques of drug susceptibility testing concerning the yeasts associated with VVC and RVVC employed by Brazilian laboratories. In this regard it is difficult to compare regional studies leading to empirical treatment. Besides, the scientific data on the epidemiology of VVC and RVVC are very limited, especially in our country.

Regional surveys are necessary to determine the involvement of different *Candida* species in the aetiology of VVC and RVVC, as well as their antifungal susceptibility patterns, since many patients do not respond to the initial therapy. The epidemiological data produced in these studies might assist in the handling of VVC and RVVC when it is impossible to confirm the clinical diagnosis through microbiological tests.

Knowledge of the drug susceptibility patterns in strains circulating in the different regions will also allow the rationalization of the empirical use of antifungal drugs, contributing to the control of the resistance phenomenon. Furthermore, these studies might be useful in predicting the recurrence of the disease.

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