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COMPARISON OF ORAL ITRACONAZOLE WITH ORAL FLUCONAZOLE IN TREATMENT OF VULVOVAGINAL CANDIDIASIS

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ABSTRACT

Introduction: Vaginal candidiasis is a common disease in women during their lifetime, during pregnancy and oral contraceptives users. Although several antifungals are routinely used for treatment; however, vaginal candidiasis is a challenge for patients and gynecologists. **Objectives:** To compare the efficacy of oral itraconazole with oral fluconazole in treatment of patients of vulvovaginal candidiasis presenting to Outpatient Department of a tertiary care hospital. **Study Design:** Randomized controlled trial. **Setting:** Department of Obstetrics and Gynaecology, Unit-3, Jinnah Hospital, Lahore. **Duration of Study With Dates:** Study was carried out over a period of six months from 17-11-2017 to 16-05-2018. **Subjects And Methods:** A total of 148 patients (74 in each group) were enrolled in this study. Group-A received Oral itraconazole (200mg twice daily for one day) and group-B administered with Oral fluconazole (150mg single dose). Results: In group-A mean age of the patients was 23.2±4.2 and in group-B 21.7±2.8 years. Married patients were 25 (33.8%) in group-A and 13 (17.6%) in group-B and remaining were unmarried. Majority of the patients belonged to lower socioeconomic status in both groups. Mean duration of disease was 1.6±0.6 and 2.2±0.7 weeks in group-A and B, respectively. In group-A 20 patients (27%) and in group-B 5 (6.8%) were diabetic. 16 patients (21.6%) of group-A and 5 patients (6.8%) of group-B used IUCD. Efficacy was found to be in 66 patients (89.2%) of group-A and 53 patients (71.6%) from group-B. Stratification with regard to age, marital status, socioeconomic status, duration of disease, diabetes status and use of IUCD was also carried out. **Conclusion:** Itraconazole was found to be more effective in the treatment of vulvovaginal candidiasis compared to fluconazole, and might represent a better choice in treating the condition.

KEYWORDS: Vulvo Vaginal Candidiasis, Oral Fluconazole, Oral Itraconazole.

INTRODUCTION

Vulvovaginal candidiasis (VVC) or Candida vaginitis is a common fungal infection among adult women during reproductive ages.[1] It is the second most common cause of vaginitis symptoms (after bacterial vaginosis) and accounts for approximately one-third of vaginitis cases.[1] It has been estimated that 75% of all adult women experience at least one period of vulvovaginal candidiasis in their lifetime.[2] Vulvovaginitis is characterized by inflammation of the vagina and the vulva. Vulvovaginal candidiasis is predominantly caused by strains of Candida albicans (>90%).[3] Candida species are commonly found in small amount in a healthy vagina. However, when an imbalance occurs, such as change in normal acidity of a vagina or the change in hormonal balance, the Candida multiplies and symptoms of candidiasis like non-specific vulvovaginal pruritus, soreness, thick curdy white vaginal discharge, and dyspareunia appear.[3] The mechanism by which Candida species transform from asymptomatic colonization to an invasive form causing symptomatic vulvovaginal disease is complex, involving host inflammatory responses and yeast virulence factors.[3]

Itraconazole is a lipophilic agent and its absorption is therefore poor and variable.[4] Itraconazole capsules should be taken with food, as their water-solubility (and hence absorption) improves when gastric pH falls. The most frequently reported side effects associated with itraconazole are gastrointestinal (abdominal pain, nausea and vomiting, dyspepsia).[5] Other side effects include dizziness, pruritus and headache. Fluconazole is water soluble and available in oral capsule, oral solution and saline-based I/V solution formulations.[6] All formulations exhibit predictable pharmacokinetics. When given orally, fluconazole is rapidly absorbed, with peak plasma levels occurring 1-3 h after dosing.[6] Absorption is unaffected by food or gastric acidity. The most common side effects are associated with the gastrointestinal tract (nausea, abdominal discomfort, vomiting, diarrhoea).[7] In the past, antifungal drug resistance was not known to exist, but today primary and

secondary antifungal drug resistance has been proved by extensive multicentre studies.[5,7] Although in vitro resistance to drug almost always mean a high rate of failure in the treatment, but in vitro sensitivity of the Candida species to antifungal drugs does not always mean successful treatment.[6,7]

My reference study, carried out by Akhter et al at Shifa Hospital Karachi, had a sample size of 60 patients with 30 patients each in both groups. Patients were assessed for vaginal discharge, itching, burning, erythema and oedema at presentation. Group 1 was treated with capsule fluconazole 150mg stat, and Group 2 with capsule itraconazole 200mg twice for one day. They were assessed for cure (cessation of all presenting symptoms) and relapse (recurrence of presenting symptoms) on day 7 and 21 respectively. At the 7th day follow-up for all the symptoms, 21 (70%) of the total in Group 1, and 15 (50%) of Group 2 had been cured completely; 7 (23.33%) patients in Group 1 and 8 (26.6%) in Group 2 showed clinical improvement; 2 (6.66%) in Group 1 and 7 (23%) in Group 2 showed no response.8 Relapse was observed in 28.5% and 53% of the cured cases with itraconazole and fluconazole respectively at day 21.[8] It was concluded that itraconazole was found to be more effective in the treatment of vulvovaginal candidiasis compared to fluconazole with high cure and low relapse rate.

Vulvovaginal candidiasis is a common problem especially in an under-developed country like Pakistan where limited resources are available. Only one published study is available regarding comparison of itraconazole and fluconazole in treating vulvovaginal candidiasis in Pakistani population. The small sample size of the reference study necessitates further studies to validate the findings. Pakistani women are living in hot and humid environment and are more prone to developing these types of infections. Furthermore it is not known whether ethnic differences affect the efficacy of these drugs. The rationale of my study will be to compare the efficacy of oral itraconazole and oral fluconazole in treating patients of vulvovaginal candidiasis so that high cure rate with low relapse rate could be achieved thereby decreasing the morbidity associated with this disease and reducing the burden of disease.

REVIEW OF LITERATURE

Vulvovaginal candidiasis (VVC)

Species from genus Candida have a wide distribution in nature and can be found in humans, domestic and wild animals as well as in diverse environments including hospitals. These yeasts belong to the normal flora of humans and can colonize the mucosal surfaces of the genital, urinary, respiratory and gastrointestinal tracts, as well as oral cavity, nails, scalp and skin.[9] However, Candida species can be commensal organisms or transform a symptomless colonization into an infection. Thus, these species are characterized as opportunistic and can change from harmless to pathogenic upon variation of the host conditions. Candida infections are mainly superficial, but in severely immunocompromised patients serious systemic infections can occur. Most, if not all women carry Candida in vagina at some point of their lives, yet without symptoms of infection.[10] Candida organisms gain access to the lower genital tract mainly from the adjacent perianal area. There is a balance between Candida organisms and vaginal defense mechanisms against Candida, such as lactobacilli and immune responses that allow the persistence of Candida species as vaginal commensals[11], thereby changes in the host vaginal/vulvar environment inflammation in the presence of Candida species and in the absence of other infectious agents.[12]

VVC can be classified into uncomplicated and complicated cases, a classification that has been accepted by several authors.[12,13]

Uncomplicated VVC is characterized by fewer than four episodes per year with mild to moderate severity caused by Candida albicans in apparently healthy women. Complicated VVC include episodes due to non-Candida albicans Candida (NCAC) species or severe cases caused by any Candida species. Moreover, recurrent VVC (RVVC), which is characterized by four or more episodes per year, and VVC in the presence of recognized risk factors (e.g. pregnancy, diabetes and immunosuppression) are also classified as complicated VVC.[12]

The clinical symptoms of VVC are nonspecific and can be associated with a variety of other vaginal diseases, such as bacterial vaginosis, trichomoniasis and gonorrhea. The most common clinical manifestations are vulvar pruritus and burning accompanied by vaginal soreness and irritation leading to dyspareunia and dysuria. Vulvar and vaginal erythema, edema and fissures are also commonly found.[14]

Epidemiology of VVC

VVC is not a reportable disease, and therefore, the information on its incidence is incomplete and based on epidemiology studies that are often hampered by inaccuracies of diagnosis and/or the use of non-representative populations.[15] VVC is considered the second most common cause of vaginitis after bacterial vaginosis.[16] It is estimated that approximately 10–15% of asymptomatic women are colonized with Candida, 70–75% of women will experience an episode of VVC in their lifetimes, 50% of initially infected women will suffer a second VVC event and 5–10% of all women will develop RVVC.[15]

Symptoms and signs of VVC are not specific to the disease[16] and the presence of Candida in the vagina is not necessarily indicative of VVC since asymptomatic women can be colonized. Therefore, the diagnosis of VVC requires correlation of clinical findings and

laboratory confirmation of Candida. The incidence of vaginal colonization by Candida species whenever asymptomatic women were also included in the study. The incidence of VVC in symptomatic women varies depending on the locations as well as the populations studied. The studies published during the last years reported incidences of the disease in symptomatic women that range from 12.1 % to 57.3 %. The highest incidences were reported by epidemiologic studies made in African countries, Nigeria[17] and Tunisia[18], with 57.3% and 48.0%, respectively), followed by Brazil[19]; and Australia.[20] The lowest incidences were reported in European countries (Greece[21] and Italy[22], with 12.1 and 19.5%, respectively) and India (17.7 to 20.4%).[23]

All these epidemiologic studies were consensual in reporting higher incidence of VVC in women at reproductive age (20–40 years) than those at menopause. Regarding asymptomatic colonization, Brazilian.[19]

Microbiology of VVC

The most common Candida species associated with VVC are C. albicans, C. glabrata, Candida tropicalis, Candida parapsilosis and Candida krusei. Typically, a single species is identified, but two or more species have been found in some women with VVC (1–10%). Most of these mixed infections are caused by an association between C. albicans and C. glabrata.[18] In fact, C. albicans is the most common species identified in women with VVC followed by C. glabrata. A recent ex vivo study demonstrated higher colonization and invasion of vaginal tissue by C. albicans than by C. glabrata, but an enhanced invasion by C. glabrata in co-infection with C. albicans.[24]

Studies reported higher association of C. albicans with VVC than NCAC species. In American[25], European[21] and Australian[26] studies C. albicans was the most common specie identified in women with the disease (70.0–89.0%), followed by C. glabrata (3.4– 20.0%). Also, Chinese[27], and Iranian[28] studies show a predominance of C. albicans (65.1–90.4%), however in some Asian and African studies Turkey.[29]

Historically, 85–95% of Candida species identified in women with VVC were C. albicans[30]; however, most studies, published during the last years, reported incidence of C. albicans below 85% and in some countries even below 50%. It has been suggested that the widespread and inappropriate use of antifungal treatments (self-medication and prolonged anti-fungal therapy) may lead to the selection of NCAC species (such as C. glabrata), which are more resistant to the commonly used antifungal agents than C. albicans.[31] In fact, NCAC species have been more commonly isolated among patients with RVVC than in women with sporadic VVC[18,21] possibly due to a higher antifungal exposure and widespread use of over the counter antimycotics among patients with RVVC. High percentages of NCAC species causing VVC, mainly C. glabrata, have been also associated with increasing age[27], patients with uncontrolled diabetes and HIV-infected women. These associations are possible due to changes in patient physiology, hormone balance and decrease in immune functions.

Compared to C. albicans, NCAC species are generally associated with higher resistance to the azoles, the most commonly prescribed class of antifungal agents. The use of nonazole antifungals, such as boric acid and flucytosine, has been shown to be effective in treating VVC caused by NCAC species, especially C. glabrata[32], which demonstrates intrinsically low susceptibility to the azoles and the ability to develop high resistance to them. The high resistance levels of NCAC species to the commonly used treatments associated with an increasing identification of these species in women with VVC highlights the importance of identifying Candida species within vaginal samples, in order to provide physicians with information concerning the proper treatment for their patients.[25]

Candida virulence factors

Until a few decades ago, it was believed that Candida microorganisms passively participated in the establishment of an opportunistic fungal infection, caused only by an organic weakness or an immunocompromised host. Today, there is consensus that these yeasts actively participate in the pathogenesis of the disease process, using mechanisms of aggression called virulence factors.[33] Thus, the pathogenicity of Candida species is mediated by a number of virulence factors that include adhesion, biofilm formation, extracellular hydrolytic enzyme production, hyphal formation and phenotypic switching.

Adhesion

Adhesion of Candida to host surfaces is required for initial colonization of human tissues, contributes to persistence of the microorganism within the host and is essential in the establishment of infection.[34] Therefore, the primary event in VVC is the adhesion of Candida species to vaginal epithelial cells. Some studies have confirmed that Candida species have the ability to adhere to this type of cells.[35]

In addition, Candida species can adhere to the surface of medical devices, often promoting device-related infections, like VVC in women using intrauterine device (IUD) as contraceptive method.[36] The initial attachment of Candida cells to biotic and abiotic surfaces is mediated by cell surface physicochemical properties and promoted by specific cell surface proteins, called adhesins.[37] Adhesins recognize host ligands, such as serum proteins and components, of the extracellular matrix of host tissues (e.g. laminin, fibronectin, collagen, vitronectin and entactin), or promote the binding to abiotic surfaces through hydrophobic interactions.[38] In C. albicans, a major group of adhesins is encoded by the agglutinin-like sequence (ALS) gene family that

comprises eight members (ALS]-7, 9).[39] Cheng et al. (2005)[40] detected expression of all ALS genes in vaginal specimens of women with VVC, but expressions of ALS], ALS2, ALS3 and ALS9 were detected more frequently than of ALS4 to ALS7. These investigators also demonstrated that the expression of C. albicans ALS genes has host-site specific influences. In C. glabrata, up to 23 different genes encoding epithelial adhesins (EPA gene family) were already identified, and three genes, EPA], EPA6 and EPA7, have been shown to encode functional adhesins.[41] Alves et al. (2014b)[24] detected expression of these three genes in reconstituted human vaginal epithelium (RHVE) infected with C. glabrata, with the highest value exhibited by EPA].

These investigators also detected all ALS genes in RHVE infected with C. albicans, with the highest expression exhibited by ALS3 and ALS6 and the lowest by ALS7. Interestingly, in RHVE co-infected with C. glabrata and C. albicans the EPA genes were down-regulated or absent and ALS genes were generally similar to those observed in single infection, with the exception of a highly increase in ALS3 expression. These results suggest that probably ALS3 but not EPA adhesins are associated with the enhanced RHVE invasion by C. glabrata observed in the presence of C. albicans. In C. parapsilosis, 11 genes were identified for putative cell wall adhesins-like proteins[42] and at least three Als proteins were identified in C. tropicalis and Candida dubliniensis.[43] However, in most NCAC species, the function of identified adhesins was still poorly studied. The absence of in vitro animal–human models highlights the importance to deepen and validate the information reported on those in vitro studies.

Biofilm formation

Adhesion of Candida cells to host epithelium or medical devices has been implicated as an early step in biofilm formation. Biofilms are structured communities of microorganisms, irreversibly attached to a surface, with a high degree of organization and a self-produced extracellular matrix.[44] Biofilms are the most prevalent growth form of microorganisms in nature, with up to 80% of all microorganisms, in the environment, existing in biofilm communities. It is also suggested that over 65% of all human infections are related to microbial biofilms.[45] Biofilm formation is an important virulence factor for Candida species as it confers unique phenotypic characteristics compared to their planktonic counterpart cells including significant resistance to antifungal agents, host defence mechanisms and physical and chemical stress.[45] Furthermore, biofilm cells exhibit metabolic cooperation, community-based regulation of gene expression and the ability to withstand the competitive pressure from other organisms.[46] The association of microorganisms into biofilms is a form of protection for their development and contributes to their survival in hostile environmental conditions.[47] Clinically, the most important phenotype of biofilms is their extraordinary resistance to the conventional antifungal therapy, which has been reported to be up to 1000-fold higher than of their planktonic counterpart cells.[46] However, the resistance mechanisms of Candida biofilms to antifungal therapy are not fully understood. It is accepted that the antifungal resistance of biofilms is a complex multifactorial phenomenon that includes alterations or overexpression of target molecules, active extrusion of antifungal agents through efflux pumps and their limited diffusion in the matrix, stress tolerance, cell density and presence of persister cells.[48] Formation of mature biofilms and subsequent production of extracellular matrix is strongly dependent on species, strains and environmental conditions.[49] Concerning the vaginal environment, Candida species can form biofilms on vaginal epithelium[50] and have high capacity to produce biofilms on IUDs promoting VVC. Candida biofilms have been studied primarily on abiotic surfaces because almost all device-related infections involve growth in the form of biofilm. Recently, biofilm formation on biotic surfaces has received some attention, and Harriott et al. (2010)[50] showed for the first time that C. albicans forms biofilms in vivo on vaginal mucosa. These investigators also reported that Candida biofilm formation on the vaginal mucosa in vivo requires regulators of biofilm formation (BCR1) and morphogenesis (EFG1), which had been previously identified as necessary genes for biofilm formation on abiotic surfaces.[51] The ability of Candida species to form biofilms on the vaginal mucosa is an important clinical issue since the recalcitrance of these biofilms to the conventional antifungal therapy may prevent complete eradication of the microorganisms from the vaginal lumen and might explain the frequent recurrence of VVC. In addition, it has been realized that many Candida infections are directly linked to biofilms in which multiple species coexist, making the therapeutic management of these infections extremely difficult. Most studies are on monospecies biofilms of Candida, and information on mixed-species Candida biofilms or Candida-bacteria combinations is still scarce.[52] Mixed-species biofilms can be difficult to both diagnose and treat, requiring complex multidrug treatment strategies. Mixed biofilms, especially Candida-bacteria biofilms, can cause a dilemma for clinicians because antimicrobials directed towards one species often facilitate non-targeted organisms to continue the infection.[52] It was reported that both Candida species and vaginal bacterial pathogens as Gardenerella vaginalis[53], which cause bacterial vaginosis (BV), have the ability to form biofilms on the vaginal mucosa. However, the formation of mixed Candida-bacteria biofilms on the vaginal mucosa is still understudied. Some studies have shown that approximately 20–34% of RVVC samples contain vaginal bacterial pathogens such as Streptococcus agalactiae and G. vaginalis[54], possibly due to mixed biofilms with Candida and these bacteria. Concerning mixed biofilm formation on IUDs, a survey on biofilms formed on those devices confirmed the presence of C. albicans as well as multiple bacterial pathogens such as S. agalactiae, Escherichia coli and

Bacteroides species. Although vaginal infections are an extremely common reason for women to seek care, there is little known about the prevalence of mixed infections, particularly VVC and BV. Recently, Rivers et al. (2011)[56] published the first article to present the prevalence of BV, VVC, yeast colonization and mixed infection. In this study, BV was diagnosed in 72.5% of the participants and VVC in 15.7%. Among women with BV, 33.1% were colonized with yeast and the overall prevalence of BV/VVC mixed infection was 4.4%. The authors suggested that the presence of infection/colonization with yeasts likely predisposes women to VVC after treatment of BV with antibiotics. In fact, some studies reveal that VVC is a common side effect of BV treatment with metronidazole or clindamycin.[56] Thus, women with BV that also have yeast residing in the vaginal ecosystem can either exhibit failure of symptom resolution from therapy targeted at one infection or development of VVC from exposure to antibiotics. The little information available on the prevalence of vaginal mixed infections is likely due to the fact that most vaginal infections are diagnosed empirically without the aid of objective data. The lack of awareness as to the extent of this problem likely leads to under recognition of mixed infections, resulting in inadequate therapy.

Extracellular hydrolytic enzyme production

Candida species secrete several hydrolytic enzymes, which play an important role in adhesion, tissue penetration, invasion and destruction of host tissues. The enzymes most frequently implicated in Candida pathogenicity are secreted aspartyl proteinases (Saps), but phospholipases, lipases and haemolysins are also involved in Candida virulence.[34] Saps facilitate adhesion to host tissues and their damage and are related with changes in the host immune response. To date, 10 SAP genes (SAP1-10) were identified in C. albicans[57], three (SAPP1-3) in C. parapsilosis and at least four (SAPT1 -4) in C. tropicalis, but in NCAC species, most of the genes remains uncharacterized. In the case of C. glabrata, only one study demonstrated ability of this species to produce proteinases, but the type of proteinase remains unknown.[58] In contrast with other types of proteinases, Saps show proteinase activity only under acid conditions (pH < 4.0).[59] This feature is important for VVC, because the vaginal environment is acidic (pH around 4), providing conditions suitable for activity of Saps. Mohandas and Ballal (2011)[60] detected higher proteinase activity in vaginal isolates than in urinary and respiratory isolates, of candidiasisinfected patients, relating Sap production and site of strain isolation. Furthermore, several studies have reported higher expression of SAPs and higher proteinase activity by Candida species isolated from women with VVC than from asymptomatic vaginal Candida carriers.[61] It has been also demonstrated that the expression of C. albicans SAP1–3 has a strong and specific correlation with VVC. Phospholipases hydrolyze one or more ester bonds in glycerophospholipids contributing to host-cell membrane damage and to the adhesion of yeasts to host tissues. Several Candida species have the ability to produce extracellular phospholipases, but this ability is highly strain dependent and NCAC species produce significantly lower levels compared with C. albicans.[62] Mohandas and Ballal (2011)[60] found a greater number of phospholipase producing strains in vaginal isolates of patients with candidiasis than in respiratory and skin isolates. In C. albicans, seven phospholipase genes have been reported (PLA, PLB1 -2, PLC1 -3 and PLD1). Naglik et al. (2003)[63] detected expression of PLB1 and PLB2 in vaginal washes of VVC infected women and of asymptomatic vaginal Candida carriers, reporting lower levels in healthy women. Furthermore, Alves et al. (2014b)[24] detected expression of PLB and PLD gene families in RHVE infected with several C. albicans strains. The highest expression level was exhibited by PLD1 indicating a potential role of this factor in RHVE damage. Lipases are involved in the hydrolysis of triacylglycerols and their activity has been associated to Candida adhesion and damage of host tissues and affects immune cells.[64] In C. albicans, lipases are coded by 10 genes (LIP1-10), similar sequences were identified in C. tropicalis and two lipase genes were detected in C. parapsilosis (CpLIP1 -2), but none in C. glabrata. These enzymes are less studied than Saps and phospholipases, especially concerning their specific association with the anatomical site of infection. Haemolysins produced by Candida species degrade haemoglobin, facilitating recovery of iron, being essential to survival and persist-ence in the host. The production of these proteins was already described in several Candida species, including C. albicans, C. glabrata, C. parapsilosis and C. tropicalis; however, the genetic expression of haemolytic activity is poorly understood.[65]

Risk factors for VVC

The vaginal flora is highly dynamic with a local microbial system. There is a balance between Candida vaginal colonization and the host environment that can be disturbed by physiological or non-physiological changes, making the colonization site favorable for the development of yeasts. Healthy women can develop VVC sporadically however this infection is often attributed to the presence of host-related and behavioral factors that disturb the vaginal environment, promoting VVC. Proposed host-related risk factors include pregnancy, hormone replacement, uncontrolled diabetes mellitus, imunosuppression, antibiotics and glucocorticoids use and genetic predispositions.[15] Behavioral risks factors for VVC include use of oral contraceptives, intrauterine device, spermicides and condoms, and also some sexual, hygienic and clothing habits.[66]

Pregnancy has been considered an important risk factor for the development of VVC because several studies report high incidence of the disease in pregnant women. Table 3 shows studies published during the last years, concerning the incidence of VVC in pregnant and

nonpregnant women. The epidemiologic studies have been consensual in reporting higher prevalence of the disease in pregnant women than in non-pregnant patients, although the incidence varies depending on the locations. In the last years, most studies of VVC incidence in pregnant women were made in India and Nigeria, probably because these countries have high birth rate and thus, have interest to study pregnant-related diseases. In Indian studies.[23,67] the incidence of VVC in pregnancy ranges between 10.0% and 76.0% and in nonpregnancy between 7.7% and 31.0%. A Nigerian study[68] reported an incidence of VVC in pregnancy and nonpregnancy of 47.7% and 20.3%, respectively, and Greek[21] and Belgian[69] studies reported a little less incidence of the disease in both conditions. In addition, since the vaginal colonization is a prerequisite for symptomatic VVC, some studies have also studied the incidence of Candida colonization in non-symptomatic pregnant women. These studies reported higher prevalence of vaginal colonization by Candida in pregnant women than in those who were not pregnant, indicating that pregnancy increases vaginal colonization.[70]

The high incidence of VVC in pregnancy has been attributed to the increase of sex hormones secretion in pregnancy. In fact, during pregnancy, VVC prevalence is higher in the last trimester, when levels of hormones are more elevated, even though symptomatic recurrences are common throughout pregnancy.[71] Furthermore, in nonpregnant women the infection is more incident during the luteal phase of the menstrual cycle, which is the phase with the highest hormone secretion. Kalo and Segal (1988)[72] also demonstrated that the level of in vitro adherence of C. albicans to human vaginal exfoliated cells (VEC) has a correlation with the hormonal status of the cell donors, being higher in VEC of pregnant women and of those in luteal phase of the menstrual cycle. The hormonal dependence of VVC is also evidenced by the fact that the disease is uncommon in pre-puberty and post-menopause, except in women taking hormone replacement therapy (HRT).[22] HRT is used to counter adverse the consequences associated with the decrease in hormones secretion in postmenopause, such as osteoporosis, diabetes mellitus, cardiovascular disease and neurodegeneration; however, it has been considered a risk factor for the development of VVC. Higher incidence of VVC has been reported in women receiving HRT (26.0–29.4%) than in post-menopausal women without HRT (4–12.6%).[73] All authors agree that the high hormonal levels of pregnant women and of those receiving HRT are the main factors responsible for the relation of those conditions with VVC. However, emotional stress, suppression of immune system and eating habits of sugar-rich food may also contribute to the development of VVC in those women.[31]

The two types of female sex hormones are estrogens and progestins. The most important progestin is progesterone, which is secreted by the corpus luteum, placenta and by adrenal cortex. During the luteal phase of the menstrual cycle, significant amounts of progesterone are secreted and during pregnancy its levels increase about 10 times. Progesterone is important in many vital actions of the woman, such as endometrial and breast development, maintenance of pregnancy, decrease in insulin action and increase in sodium excretion by the kidneys.[74] The main estrogen in pre-menopause is R-estradiol and in postmenopause is estrona, which has a lower estrogenic potency than R-estradiol. Significant amounts of estrogens are secreted by the ovaries and during pregnancy high amounts of these hormones are secreted by the placenta increasing about 30 times their levels. Estrogens perform many essential actions in women, including the development of secondary sex characteristics, uterine growth and conservation of the vaginal mucosa.[74] Despite being accepted that VVC has a hormonal dependency, the mechanisms by which progesterone and estrogens act in the disease are not fully understood. One proposed mechanism is associated with the increase in glycogen load in vaginal epithelium when the levels of progesterone and estrogens increase. The walls of vagina are lined with the pavement of epithelial cells, which produce glycogen in proportion to hormonal levels, and thus the state of the vaginal mucosa reflects female hormonal status in different lifetime stages.[73] The production of glycogen by hormone stimulated epithelium possibly contributes to the proliferation of Candida species when host hormones exceed a certain level, because glycogen provides an excellent nutritional source of carbon for Candida growth.[75] In addition to the effect on vaginal epithelium, it has been also proposed that sex hormones inhibit aspects of both innate and adaptive immunity at systemic or local level. In fact, studies in vitro with vaginal epithelial cells found that progesterone and estrogens inhibit Candida-specific human peripheral blood lymphocyte (PBL) responses[76] and that estradiol significantly reduces antimicrobials production (HBD2 and elafin). Furthermore, analyses of cervical–vaginal secretions demonstrate that chemokines and cytokines (IL-6 and IL-8), antimicrobials (HBD2 and lactoferrin) and levels of IgA and IgG antibodies are depressed by 10- to 100-fold at mid-cycle, remain depressed for 7–10 days and rise to proliferative levels in the end of the menstrual cycle.[77] In addition, Nohmi et al. (1995)[78] reported that physiological blood level of progesterone of pregnant women clearly suppresses mice neutrophil anti-Candida activity.

Oral contraceptives

Some studies have shown that women using oral contraceptive more often get recurrences of vulvovaginal candidiasis compared with non-users, while oral contraceptives (OC) per se do not seem to affect the occurrence of sporadic WC.[79] Historically, the data are not conclusive. Frequent sexual intercourse in OC users may also contribute to the activation of Candida infection. Women using OC have been shown to have or changes in plasma insulin and glucose tolerance,

possibly an effect of progestogen. There is no evidence of an increase in manifest diabetes mellitus due to the use of OC.[80]

Sexual and hygiene habits

The role of sexual habits in the activation of candida has been evaluated. Frequent sexual intercourse[81], several lifetime sexual partners, and oral sex have been suspected to increase the risk for developing recurrent vulvovaginal candidiasis. The use of intrauterine device has also been discussed.[82] However, no consistent results have been presented. Allergy to semen of the partner or products in the semen derived from what the partner may have ingested, has been reported in a few cases.[83]

Hygiene habits that are associated with RWC include repeated cleansing as well as shaving of the genital area, vaginal douching, and the use of soaps low in pH[80], which will cause drying out of the vulvar mucosa and micro lesions in the skin barrier. The use of panty-liners is associated with the existence of RWC.[66] It is however not clear if these hygiene habits are possible causes for developing RWC, or consequences of the discomfort of having repeated vulvovaginal complaints.

Glucose control

Often, women with RWC are advised to abstain from carbohydrates, as a prophylactic aim. It is known that insulin-dependent diabetics with poorly regulated glucose levels easily will develop vulvovaginal candidiasis. Yeast grows in vitro in a sugar-rich environment. However, in the literature there exists very little scientific evidence for the beneficial effect of a carbohydrate low diet. Horowitz found elevated levels of glucose, arabinose, and ribose in the urine of subjects with recurrent and ongoing vulvovaginal candidiasis.[84]

Establishment and Dissemination of Vaginal Candidiasis

Colonization of the epithelial cells is the first step in the establishment of vulvovaginal candidiasis. Adherence to the host epithelial cells is accomplished either through the use of adhesins or the involvement of proteases, which do not necessarily have to be enzymatically active in order to be involved in a pathogen-host docking event.[85] Although hyphal forms are commonly associated with disease and blastospores with benign colonization, blastospores have also been isolated from disease sites.[86]

In order to cross tissue planes and establish an invasive infection, Candida albicans must invade cells that are not normally phagocytic, such as epithelial and endothelial cells. A common histopathologic finding in all types of candidiasis is the presence of fungal cells within these tissues. The mechanism by which this invasion occurs has been the focus of intense investigation for many years.[87] Two methods of cellular invasion have been established in vitro; invasion through hydrolytic degradation of the targeted cell wall and the induction of normally nonphagocytic cells to engulf the pathogen.[88] Recent work by Filler et al showed that the yeast adhesin Als3 binds to either a N-cadherin or an E-cadherin of the host epithelial cell resulting in the fungal cell being phagocytized.[89]

Invasion into the primary layer of cells via enzymatic digestion is more characteristic of filamentous Candida due to their upregulation of SAPs (secreted aspartyl proteases), while blastospore entry appears to primarily occur through the induction of phagocytosis.[89] Candida albicans contains a myriad of proteases, of which only the aspartyl protease group is secreted. These SAPs exhibit a broad range of pH optima and substrate specificities thereby making them potent virulence factors.[85] Once the pathogen has entered the primary infection cells, it is the hyphal form that is predominantly responsible for tissue penetration and deeper tissue invasion. Via its filamentous forms, C. albicans is able to progress into the host tissue through further cell lysis and secondary blastospore budding.[85]

If the pathogen is able to cross through the vaginal tissue, entry into the bloodstream can occur and a disseminated infection can be established. Once in the host bloodstream, C. albicans is readily able to migrate to other tissues and organs and establish a widespread systemic infection. The stages of infection and the resulting establishment of systemic infection are summarized below. It should be noted that a systemic Candida albicans infection poses a significant health risk with a mortality rate of approximately 50%.[90]

Host Defense against Vaginal Candidiasis

As a result of the staggering numbers of Candida albicans infections per year, the majority of all healthy adults have developed a Candida-specific adaptive immunity. This adaptive was immunity is demonstrated by the presence of serum/mucosal antibodies, in vitro T-cell responses, and delayed skin test reactivity.[92] What role these adaptive immune responses play in the prevention of or protection against Candida infections of the urogenital tract is not fully known, but the latest experimental findings show that their contributions appear to be nominal.[91]

Over the past 20 years, there have been innumerable studies pertaining to host-pathogen interactions and the mechanisms of urogenital infections by Candida albicans. Despite the large number of studies undertaken to identify the protective host mechanisms against vaginitis we still do not have a complete understanding of how our immune system deals with potentially pathogenic commensals of the urogenital tract.

Mucosal candidiasis includes orapharyngeal, esophageal, gastrointestinal, and vaginal infections.[92] Prior to the AIDS epidemic, researchers thought that all mucosal membranes were equally susceptible to Candida

infection through the same mechanism.[92] A large percentage of AIDS patients suffering from T-cell immuno-suppression developed mucosal candidiasis (mainly orapharyngeal in nature), and experimental models showed a strong role for T cells against Candida infection. Clinical studies and animal models investigating RVVC showed no role for systemic or local cell-mediated immunity (CMI) or a shift in a Candida-specific Th1 to a Th2 response at the vaginal mucosa.[93] The lack of a systemic immune response at the urogenital tract against Candida is further backed by the observation that although female AIDS patients are commonly infected with oral candidiasis, vaginal candidiasis was no more prevalent than in the healthy population.[94]

Puzzled as to why there was a lack of a T-cell response at the vaginal mucosa although a Candida-specific Th1-type immunity was evident in the blood and draining lymph nodes in mice, Fidel et al.[94] began to research the mechanism that was preventing CMI from protecting against vaginal candidiasis. Their resulting experiments revealed that a strong down regulatory cytokine, TGF-ß, was constitutively present at the vaginal mucosa and its expression and secretion by vaginal epithelial cells was transiently increased in response to either an infection or estrogen. Other cytokines that affect Th1/Th2 cells were extremely low during infection. From this new study, the current paradigm of immunoregulation emerged as an explanation for the apparent lack of CMI protection against vaginal infection. Fidel has proposed that since Candida albicans is a vaginal commensal, the evolution of immunoregulation to avoid chronic inflammation at the reproductive tissue of the host, would only strengthen their symbiotic relationship.[95]

Investigations into the possible role of innate immunity at the vaginal mucosa showed that there was no detectable correlation between the presence of polymorphonuclear neutrophils (PMNs) and natural killer cells (NKs) and a response to an infection.[92] However, during these experiments, Fidel et al. discovered that vaginal epithelial cells from mice, humans, and macaques have the ability to inhibit C. albicans growth. This inhibitory activity was elucidated to be an acid-labile protein bound to the vaginal epithelial cell wall that upon contact with Candida albicans causes an unknown intracellular event resulting in the inhibition of its growth.[96] This activity was also evident in oral epithelial cells. Since this activity is fungistatic and not fungicidal, it may provide evidence of a host mechanism to control commensalism. Clinical studies comparing the role of this fungistatic mechanism in healthy and RVVC afflicted women revealed that there was only a minor reduction in fungistatic activity in those with RVVC (Nomanbhoy). Since the reduction of activity was minor, researchers believe that there are other undiscovered host factors that are responsible for protection against fungal vaginitis.[96]

Composition of Cervical-Vaginal Fluid

The mucosal fluid covering the vaginal epithelium is primarily made up of endometrial and oviductal fluids, secretions from cervical vestibular glands, and plasma transudate.

The molecular components of the fluid include inorganic salts, urea, amino acids, proteins, and a number of fatty acids; the fatty acids primarily provided by commensal organisms, of which Lactobacillus spp. predominate (Tang). Recently, the proteome of the cervical-vaginal fluid (CVF) of lavages obtained from healthy and Candida-colonized vaginas has been elucidated. Tang et al. discovered that there were surprisingly high levels of normal serum proteins present in the cervical-vaginal fluid. The serum proteins, albumin, immunoglobulin chains, and transferrin were found in relative abundance and accounted for 47% of all proteins identified within the fluid (Tang). Another startling discovery was the absence of proteins associated with vaginal commensal bacteria. Out of the 147 proteins identified, only one protein, an oligopeptide/dipeptide ABC transporter of Lactobacillus reuteri was identified.[97]

A further examination of the protein maps generated by Tang et al.[97] showed a clear increase in the amounts of serum proteins in lavages obtained from Candida-infected patients compared to that of healthy patients. Although the levels of serum proteins found in the lavages from colonized patients increased, the proportions of other non-serum protein components were not significantly altered. In comparison to fluids from other mucosal membranes, such as the oral and nasal passages, only 16 proteins are conserved.[97] If these proteins, or factors associated with them, play a role in host-commensal/pathogen regulation it may account for the difference seen in the human immune response against Candida albicans between these two mucosal environments.

Diagnostic methods

A careful clinical examination of the vulva and the vagina. is necessary for an accurate diagnosis. Differential diagnoses such as bacterial vaginosis (co-existence is common), condyloma, herpes genitalis, or irritative dermatitis should be ruled out.

The most common method to diagnose vulvovaginal candidiasis is by a wet mount However, hyphae are detectable in only 60 % of cases with ongoing vulvovaginal candidiasis (i.e. the sensitivity of the test).[98] Culture from vaginal samples, placed on Sabouraud and CHROMagar®, is a more reliable method with a sensitivity of approximately 90 %. PCR-detection of Candida strains has promising sensitivity and is under development, but is not yet in clinical use. Vaginal yeast culture is recommended in recurrent cases, when there are no signs in wet mount, when information of species of yeast is needed and when antifungal resistance is suspected.[99]

Glucose Metabolism

Since poorly controlled diabetes mellitus is a known risk factor for developing recurrent vulvovaginal candida infections[100], there is a belief that dietary factors like excessive intake of carbohydrates or sweets might increase the risk of recurrent vulvovaginal candidiasis. However, there are few scientific reports concerning this topic. According to one study, women with recurrent vulvovaginal candidiasis have elevated urinary secretion of glucose during ongoing infections.[101] Another study suggested a slightly impaired plasm glucose tolerance in women with recurrent vulvovaginal candidiasis.[100] However, levels of glucose in vaginal secretions have not previously been measured.

In case of severe infections such as sepsis, bacterial infections may have an influence on the glucose metabolism in healthy individuals. The glucose level will initially rise, followed by a short period of hypoglycaemia, and eventually return to normal levels.[102] In diabetic subjects, the control of glucose in plasma is more difficult, due to insulin resistance. Diabetics are more prone to higher morbidity and mortality secondary to a severe bacterial infection. This fact is possibly due to immunologic changes, in particular an impaired innate response of the polymorphonuclear leukocytes.[103] The B- and T-cell function in diabetic subjects with well-controlled diabetes is normal, however.[104] A high level of HbA1C has been associated with vulvovaginal colonization of candida in diabetic subjects. In another study, it was observed that diabetic women who were orally colonized with candida had higher oral glucose levels than diabetics and healthy control women without oral candida.[105] Although the occurrence of vulvovaginal candidiasis (VVC) in diabetic subjects has been studied, previous measurements of glucose in vaginal secretions have not been made. This thesis further investigates the levels of glucose in plasma and in vaginal secretions during oral glucose tolerance testing in women with RWC.[106]

Medical diagnosis of VVC infection is made by the presence of clinical symptoms, evaluation of vaginal pH, microscopic examination and an amine or whiff test. In VVC infection, vaginal pH typically remains normal at less than 4.5, unlike bacterial vaginosis and trichomoniasis, which causes vaginal pH to rise to greater than 4.5.[107] Other causes of an alkaline vaginal environment include menstruation, ovulation, recent intercourse, douching and infections.[108]

Presenting with an odorless discharge is a distinguishing factor of fungal infections. In the amine or whiff test, a drop of 10 percent potassium hydroxide is added to the vaginal secretions. Detecting a fishy odor can differentiate a bacterial infection from a fungal infection. Observing vaginal discharge in a saline solution (wetmount test) or a 10 percent potassium hydroxide preparation under a microscope can help confirm diagnosis of fungal infection. Under the microscope, yeast blastospores and pseudohyphae are characteristic in symptomatic patients.[109]

Being as many women have yeast as part of normal vaginal flora, microscopic evaluation and cultures may have limited value in those who are asymptomatic. However, any female patient presenting with symptoms of VVC for the first time should always be evaluated by a clinician prior to treatment with a nonprescription antifungal.[108]

All clinicians should feel comfortable talking with female patients about their symptoms in order to eliminate the potential of misdiagnosis or treatment of another form of vaginitis or allowing an underlying condition to go untreated. In order to determine appropriate treatment, clinicians should ask patients specific questions about their discharge and associated symptoms. Questions about discharge should include presence of odor, blood, on-set, duration, amount and previous episodes. Inquiring about associated symptoms should include complaints of itching, burning, fever and pelvic, abdominal or shoulder pain. Asking about age, pregnancy status and medical, sexual and medication history also should be part of the evaluation. Ideally, pregnant patients should consult with their practitioner, however, nonprescription antifungal in the appropriate dose, formulation and length of treatment are usually an option for this patient population. Other vaginal conditions, which are noninfectious, could be confused with the symptoms of VVC—specifically complaints of vaginal itching and irritation. These symptoms could be a result of a product hypersensitivity or allergy to latex condoms, spermicides, jellies, use of scented feminine products or frequent douching. These symptoms require a different treatment regimen.[108]

Pharmacological Treatment

The goals of treating VVC infection include relief of symptoms, eradication of infection, re-establishing normal flora and prevention of recurrence. The desired time frame of use of nonprescription antifungals is symptomatic relief within three days, eradication of infection within seven days and no recurrence within two months. Products used in the treatment of noninfectious vaginal irritation should provide relief within a few days, but should not be used for more than seven days.[110]

Vaginal antifungals

There currently are four nonprescription vaginal antifungals available in the imidazole class, including butoconazole, clotrimazole, miconazole and tioconazole. Clotrimazole was the first azole brought to the market in 1990, followed by miconazole, butoconazole and tioconazole. The antifungals work by altering and damaging the fungi membrane, resulting in the death of the organism.[110]

Small amounts of the topical antifungals are systemically absorbed, and higher concentrations of the azoles can remain in the vaginal area for longer periods of time. This is observed with the higher concentrations of the one-day preparations.[111]

All vaginal antifungals are approved for the treatment of vaginal candidiasis. The products available as combination preparation have an additional indication for the relief of external itching and irritation due to the infection (i.e., Monistat-1 combo, Mycelex-7 combo). These preparations contain a small tube of antifungal cream and would benefit those patients with complaints of external itching, as well as with internal irritation and itching. The side effect profile of these products is well tolerated with mild itching, irritation and burning. It is important to explain to the patients that these symptoms are side effects of the medication and not a worsening of their condition. Patients may have a tendency to discontinue their medication if they observe a worsening of their symptoms after the start of their treatment. The only product with a drug interaction warning is the ingredient miconazole, which states an increased risk of bleeding in patients taking warfarin or other anticoagulants. Caution should be used in those patients to determine the most appropriate therapy.[112]

Although sexual intercourse during treatment of VVC is discouraged, women who do engage in intercourse should use precaution when using condoms or diaphragms during treatment with a vaginal antifungal. Because of the mineral oil contained in these preparations, barrier methods of contraception could be damaged resulting in unexpected pregnancy or exposure to STDs. Clotrimazole is the only azole that does not contain this particular warning as it does not contain mineral oil as an active ingredient. All vaginal antifungal product labels are required by the Food and Drug Administration to include a warning regarding recurrence of symptoms within two months, use during pregnancy or any serious underlying medication condition, such as diabetes and HIV/AIDS.[112]

Vaginal antifungals are available as one-day, three-day or seven-day preparations. They also are available in several formulations, including cream, ointment and ovule, sometimes referred to as vaginal inserts or suppositories. Currently, tioconazole is the only vaginal antifungal available as an ointment formulation, and its higher concentration allows for oneday use. The cream-based products may be purchased as prefilled, disposable or reusable applicators. Product selection for the antifungals is based on cost, length of treatment, formulation, convenience and ease of use. The price does vary among the products depending on the type of formulation and length of treatment, however, all products except butoconazole are available in generic.[113]

A common question is which length of therapy should the patient choose, and is one better than the other. Numerous studies have concluded that efficacy among one-, three- and seven day preparations is comparable in providing symptomatic relief within three days and eradication within seven days.4 Approximately 80 percent to 90 percent of patients will have relief of symptoms and cure of infection with any of the available nonprescription antifungal.[113] Therefore, overall product selection is based on patient preference and convenience of the chosen treatment, which is typically the one- and three-day preparations. According to the CDC, the only specific product recommendation is made for pregnant women who should use a seven-day product because they provide a lower concentration of medication over several days.[114]

Whether to use a cream or ovule is another factor to consider when selecting a vaginal antifungal. Each formulation has advantages and disadvantages. When choosing among the cream formulations, patients have the choice of various applicators, such as prefilled medication applicators, which provide additional convenience so the patient does not have to touch the cream. The reusable applicators should be washed with soap and water to prevent reinfection, therefore prefilled applicators tend to be preferred. Creams should be used at bedtime with a minipad to prevent any leaked medication from staining bedclothes or sheets. In comparison, vaginal inserts or ovules allow for daytime administration with less incidence of staining. This formulation is more convenient for patients, but studies have shown comparable efficacy between the ovule and cream products.

In a study by Upmalis et al, a singledose miconazole ovule was compared with seven-day miconazole cream in patients with uncomplicated vaginal candidiasis. Overall, cure rates were comparable between the ovule and miconazole cream (i.e., 71.7 percent versus 70.1 percent, respectively).[115]

The time to symptom relief and complete relief was significantly higher in the ovule group compared with the cream (i.e., four versus five days, p=0.008 and three versus four days, p=0.025, respectively). However, overall safety results were comparable. The products were concluded to be equally efficacious, with the ovule providing additional convenience and was therefore the preferred treatment among patients. Fluconazole is a commonly prescribed one-day oral dose prescription medication used in the treatment of VVC infection. Numerous studies have concluded similar safety and efficacy between the use of oral fluconazole and topical nonprescription antifungals.[115]

In a study by Sobel et al, a single dose fluconazole was compared with a seven-day intravaginal clotrimazole therapy in patients with uncomplicated VVC. There were no clinically significant differences in the cure rates

observed among the two groups. Although additional side effects were reported with the oral treatment, both regimens were equally efficacious. A health care provider seeing a patient with a first VVC infection should base medical treatment on drug costs, convenience, patient preference and side effect profiles.[116]

Additional counseling points during treatment with vaginal antifungals includes avoiding sexual intercourse, avoiding douching to prevent washing out medication, no use of tampons (which may absorb medication), no use of condoms or diaphragms (which may become damaged and ineffective), the ability to use during menstrual flow and that patients should continue to use for full treatment duration even if symptoms improve.

Recurrent VVC

Recurrent episodes of VVC respond well to a longer duration (i.e., seven to 14 days) of therapy of either oral (i.e., fluconazole) or topical azole therapy for acute infection.[117]

After the acute episode has been treated, it is then recommended to initiate maintenance for approximately six months with medications including ketoconazole, terconazole, prescription butoconazole (Gynazole-1) or itraconazole.[118] Patients who have uncontrolled diabetes, are immunosuppressed, present with treatment resistance to C. albicans or with non-albicans type may be candidates for maintenance therapy. Unfortunately, it is estimated that 30 percent to 40 percent of patients will develop recurrences once therapy is discontinued.[118]

One study evaluating maintenance regimens included a randomization of patients with recurrent VVC to placebo group, 400 mg ketoconazole for five days after menses for six months or 100 mg ketoconazole daily for six months. At the six month follow-up, recurrence rates were significantly lower in the daily ketoconazole group compared with the 400 mg group and placebo (i.e., 5 percent, p=0.001 versus 29 percent, p=0.01 versus 71 percent).[110] The study also observed high relapse rates after discontinuation of maintenance therapy with high-dose (43 percent, p=0.05) and low-dose ketoconazole (52.4 percent, p=0.05). Patients who require longer maintenance therapy should be evaluated to determine the most appropriate regimen, taking into consideration drug cost, side effect profile, cost of monitoring, drug interactions, adherence concerns and ease of administration.[110]

Vaginal antipuritics

Patients may present with noninfectious vaginal irritation that is the result of inadequate hygiene or allergy to vaginal products. Symptoms may mimic VVC, however the predominant symptom is external itching, soreness and discharge that resembles the physiologic type. Patients should not use these products for more than one week and should be re-evaluated if symptoms worsen or do not improve with treatment. Ingredients used for vaginal irritation include anesthetics (i.e., benzocaine), external analgesics (i.e., resorcinol) and antipuritic (i.e., hydrocortisone).[110] Povidone- iodine is another ingredient found in various douche formulations, which may have bactericidal and fungicidal properties. Because of the potential risks of excessive douching, this may not be a preferred product. Frequent douching can reduce the amount of normal flora and increase a patient's risk of developing VVC, bacterial vaginosis, as well as other gynecological problems. Vitamin A, D, E and aloe vera are additional ingredients that may be found in combination products that relieve the symptoms associated with vaginal irritation.

Complementary therapies

Alternative therapies, such as boric acid, lactobacillus acidophilus and homeopathic remedies also may have benefit in treatment of itching and irritation associated with VVC infection. Lactobacillus is a probiotic, which is a micro-organism that has activity against pathogens living within the body. In VVC infection, lactobacillus works by re-establishing normal vaginal flora and inhibiting overgrowth of Candida. Lactobacillus can be found in oral supplements or in dietary sources, such as yogurt with live cultures or milk fortified with lactobacillus. Because of limited studies, there is conflicting data on whether lactobacillus taken prophylactically can prevent a VVC infection.

A study by Pirotta et al researched the effects of lactobacillus in the prevention of post-antibiotic VVC.[119] Participants took a 10-day course (six days during antibiotic treatment and four post-treatment) of either oral or vaginal lactobacillus or placebo. Symptoms of post-antibiotic VVC and microscopic evaluation of VVC were the primary outcome measurements at day.[119] Overall, the use of lactobacillus was not supported by this study, and more patients would be required to detect any significant differences among the treatment groups.[116] Other studies have shown some benefit. However, because of the small number of participants in these studies, the clinical significance remains questionable.

Additional research is warranted before specific recommendations for use of lactobacillus can be made. In the meantime, clinicians should explain to patients that lactobacillus is part of the normal vagina flora and although it may not prevent infection, it will not be harmful and it can reestablish the normal flora. Homeopathic products also are available for the relief of symptoms associated with VVC or noninfectious vaginal irritation. Homeopathic medicine uses the theory of treating certain conditions with very small diluted doses of similar natural substances. Because of the limited data on the benefits and safety of these products, patients should use the same precautions when using homeopathic remedies as with other natural vaginal preparations. Boric acid is a natural remedy that may

provide benefit in the prevention of recurrent VVC episodes and the associated symptoms of itching and irritation. Boric acid works to acidify the vagina and help restore natural lactobacillus by vaginally inserting a 600 mg suppository.[116] Other ingredients found in boric acid preparations include such herbs as Oregon grape root and calendula, which may help with irritation, inflammation and repairing of the tissues of the vagina. Boric acid suppositories should not be used during pregnancy or on damaged or irritated skin.

Guaschino et al studied the use of boric acid in comparison with oral itraconazole in patients with recurrent VVC. The study concluded that there was no clinically significant difference among treatment groups with respect to symptomatic improvement and mycological cure rates.[120] The authors did observe relapse of VVC infection following treatment discontinuation in both groups, which is common in patients with recurrent episodes. Tea tree oil, cinnamon, carvacrol and garlic are examples of herbs that may provide benefit to women with VVC infection. When counseling on natural or herbal remedies, clinicians should explain to patients that these products are not evaluated by the FDA and are not meant to treat, prevent or cure disease.

OBJECTIVES

Objective of the study was

- To compare the efficacy of oral itraconazole with oral fluconazole in treatment of patients of vulvovaginal candidiasis presenting to Out-patient Department of a tertiary care hospital.

operational definitions

Vulvoaginal candidiasis: Defined as the demonstration of a species of Candida with a potassium hydroxide (KOH) preparation on a high-vaginal-swab-smear along with the presence of 2 or more of the following clinical symptoms: vulvovaginal pruritus, soreness, scanty thick curdy white vaginal discharge, dyspareunia.

Efficacy: Defined as cessation of all clinical symptoms at day 7 after treatment.

MATERIAL AND METHODS

STUDY design

Randomized controlled trial.

Setting

Department of Obstetrics and Gynaecology, Unit-3, Jinnah Hospital, Lahore.

Duration of Study With Dates

Study was carried out over a period of six months from 17-11-2017 to 16-05-2018.

Sample Size

Sample size calculated with significance level of 5%, power of study of 80% taking efficacy in group-A (Itraconazole) of 70% and efficacy in group-B (fluconazole) of 50%.[8] Required sample 148 (74 in each group).

Sampling Technique

Non-probability consecutive sampling.

Sample Selection

Inclusion Criteria

1. Patients of vulvovaginal candidiasis as per operational definition.
2. Patients aged 18-40 years.
3. Patients who give written informed consent willingly.

Exclusion Criteria

1. Patients with other infectious causes of vulvovaginitis (e.g., bacterial vaginosis, Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoea, Herpes simplex, or human papilloma virus) assessed by a negative microscopy and gram staining for these organisms an a high-vaginal-swab smear.
2. Patients with previous history of topical treatment for vaginal infection or using other oral antifungals concomitantly.
3. Patients allergic to itraconazole and/or fluconazole

Data Collection Procedure

Data were collected after approval from Medical Ethics Committee of AIMC/JHL. Selection of sample was done from patients presenting to Outpatient department, Gynecology Unit-III, Jinnah Hospital, Lahore. Written informed consent was taken from participants. Demographic information e.g. age, sex, address, socioeconomic status, duration of disease, marital status, diabetic status, use of Intrauterine contraceptive devices etc. was noted in the proforma (attached). Single-blind was applied to reduce bias. Patients were randomized by lottery method into two groups (as shown below). All subjects were followed up 7 days after treatment; final outcome (efficacy) was recorded in predesigned proforma (attached).

Group-A: Oral Itraconazole (200mg Twice Daily For One Day)

Group-B: Oral Fluconazole (150mg Single Dose)

Data Analysis Procedure

Data was entered in SPSS version 20. Mean and standard deviation was calculated for quantitative variables (age, duration of disease). Frequency and percentage was calculated for qualitative variables (efficacy, sex, socioeconomic status, marital status, diabetes, contraceptive device use). Effect modifiers and confounders (age, socioeconomic status, duration of disease, marital status, diabetic status, use intrauterine contraceptive devices) were controlled through stratification. Chi square test was applied by taking $p \leq 0.05$, post-stratification.

RESULTS

A total of 148 patients were included in the study during the study period of six months from 17-11-2017 to 16-05-2018.

Group-A received Oral itraconazole (200mg twice daily for one day) and group-B administered with Oral fluconazole (150mg single dose).

In group-A mean age of the patients was 23.2±4.2 and in group-B 21.7±2.8 years. Married patients were 25 (33.8%) in group-A and 13 (17.6%) in group-B and remaining were unmarried. Majority of the patients belonged to lower socioeconomic status in both groups. Mean duration of disease was 1.6±0.6 and 2.2±0.7 weeks in group-A and B, respectively. In group-A 20 patients (27%) and in group-B 5 (6.8%) were diabetic. 16 patients (21.6%) of group-A and 5 patients (6.8%) of group-B used IUCD. Efficacy was found to be in 66 patients (89.2%) of group-A and 53 patients (71.6%) from group-B. Stratification with regard to age, marital status, socioeconomic status, duration of disease, diabetes status and use of IUCD was also carried out.

Table 1: Distribution of patients by age.

Age (Year)	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
18-25	57	77.0	66	89.2
26-35	17	23.0	08	10.8
Total	**74**	**100.0**	**74**	**100.0**
Mean±SD	**23.2±4.2**		**21.7±2.8**	

Table 2: Distribution of patients by marital status.

Marital status	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
Married	25	33.8	13	17.6
Unmarried	49	66.2	61	82.4
Total	**74**	**100.0**	**74**	**100.0**

Table 3: Distribution of patients by socioeconomic status.

Socioeconomic status	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
Lower	62	83.8	66	89.2
Middle	10	13.5	06	08.1
Higher	02	02.7	02	02.7
Total	**74**	**100.0**	**74**	**100.0**

Table 4: Distribution of patients by duration of disease.

Duration (weeks)	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
1	30	40.5	12	16.2
2	38	51.4	34	46.0
3	06	08.1	28	37.8
Total	**74**	**100.0**	**74**	**100.0**
Mean±SD	**1.6±0.6**		**2.2±0.7**	

Table 5: Distribution of patients by diabetes status.

Diabetes status	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
Diabetic	20	27.0	05	06.8
Non-diabetic	54	73.0	69	93.2
Total	**74**	**100.0**	**74**	**100.0**

Table 6: Distribution of patients by IUCD use.

IUCD use	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
Yes	16	21.6	05	06.8
No	58	78.4	69	93.2
Total	**74**	**100.0**	**74**	**100.0**

Table7: Distribution of patients by efficacy.

Efficacy	Group-A (Itraconazole)		Group-B (Fluconazole)	
	No.	%	No.	%
Yes	66	89.2	53	71.6
No	08	10.8	21	28.4
Total	**74**	**100.0**	**74**	**100.0**

Chi square 7.248
P value 0.007

Table 8: Stratification of age with regard to efficacy.

Age (Year)	Group	Efficacy		Total	P value
		Yes	No		
18-25	Group-A	50	7	57	0.090
	Group-B	50	16	66	
Total		**100**	**23**	**123**	
26-35	Group-A	16	1	17	0.002
	Group-B	3	5	08	
Total		**19**	**6**	**25**	

Table 9: Stratification of marital status with regard to efficacy.

Marital status	Group	Efficacy		Total	P value
		Yes	No		
Married	Group-A	20	5	25	0.825
	Group-B	10	3	13	
Total		**30**	**8**	**38**	
Unmarried	Group-A	46	3	49	0.002
	Group-B	43	18	61	
Total		**89**	**21**	**110**	

Table 10: Stratification of socioeconomic status with regard to efficacy.

Socioeconomic status	Group	Efficacy		Total	P value
		Yes	No		
Lower	Group-A	54	8	62	0.028
	Group-B	47	19	66	
Total		**101**	**27**	**128**	
Middle	Group-A	10	0	10	0.051
	Group-B	4	2	6	
Total		**14**	**2**	**16**	
High	Group-A	2	0	2	*
	Group-B	2	0	2	
Total		**4**	**0**	**4**	

*No statistics is computed because efficacy is constant

Table 11: Stratification of duration of disease with regard to efficacy.

Duration of disease (week)	Group	Efficacy		Total	P value
		Yes	No		
1 week	Group-A	27	3	30	0.209
	Group-B	9	3	12	
Total		**36**	**6**	**42**	
2 week	Group-A	33	5	38	0.090
	Group-B	24	10	34	
Total		**57**	**15**	**72**	
3 week	Group-A	6	0	6	0.134
	Group-B	20	8	28	
Total		**26**	**8**	**34**	

Table 12: Stratification of diabetes status with regard to efficacy.

Diabetes status	Group	Efficacy		Total	P value
		Yes	No		
Diabetic	Group-A	17	3	20	0.211
	Group-B	3	2	5	
Total		**20**	**5**	**25**	
Non-diabetic	Group-A	49	5	54	0.011
	Group-B	50	19	69	
Total		**99**	**24**	**123**	

Table-13: Stratification of IUCD used with regard to efficacy.

IUCD use	Group	Efficacy		Total	P value
		Yes	No		
Yes	Group-A	12	4	16	0.214
	Group-B	5	0	5	
Total		**17**	**4**	**21**	
No	Group-A	54	4	58	0.001
	Group-B	48	21	69	
Total		**102**	**25**	**127**	

DISCUSSION

Vulvovaginal candidiasis is the second most common cause of vaginitis after anaerobic bacterial vaginosis. It is observed that C. albicans accounts for 70–90% of VVC cases, with a recent emergence of non-albicans species.[121]

Itraconazole and fluconazole are safe, broad-spectrum antifungal drugs which have gained an important place in the treatment of vulvovaginal candidiasis. Their safety and efficacy have been evaluated in a number of comparative and non-comparative trials conducted in different areas of the world.[4,122]

Eradication rate observed in our study was similar to that in the literature. Our results showed efficacy with itraconazole (89.2%) and fluconazole (71.6%) are in consistent with a study conducted by Woolley PD and Higgins.[123]

Another study established the relationship of clinical outcome of candidal infection and in vitro results by the determination of minimum inhibitory concentration (MIC) of itraconazole and fluconazole.[124]

Clinically, itraconazole was effective in 64.3% of the cases, while fluconazole was effective in 71.0%. A meta-analysis[125] on various studies conducted on the efficacy of single-day dose of fluconazole comprising 3279 patients, found a positive clinical response in 94% with a range of 88-100%. Furthermore, in a similar European multicentre study, 70% patients were cured clinically during therapy and 24% improved clinically[125] Our findings do not coincide with the results of above-mentioned studies.

This difference between the efficacy of itraconazole and fluconazole could be explained by the fact that in our local setup, women might be suffering from fluconazole-resistant strain of Candida species. Identification of different strains of Candida was not included in our study. Literature also suggests that Candida Glabrata and Candida Krusie are often non-responsive to fluconazole, but are susceptible to itraconazole. Primary resistance of local candida species to fluconazole and secondary drug resistance to fluconazole, a commonly prescribed drug in our population for vulvovaginal candidiasis, cannot be ruled out. However, more local studies are required to be done for the evaluation of therapeutic efficacy of these antifungal drugs.[5,6]

CONCLUSION

Itraconazole was found to be more effective in the treatment of vulvovaginal candidiasis compared to fluconazole, and might represent a better choice in treating the condition. The small sample size of our study necessitates further studies to validate the findings.

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