CLINICOMYCOLOGICAL STUDY OF VULVOVAGINAL CANDIDIASIS

DISSERTATION

SUBMITTED FOR THE AWARD OF

M.D.DEGREE EXAMINATION

(DERMATO-VENEREO-LEPROLOGY)

APRIL 2015

THE TAMILNADU

DR. M.G.R MEDICAL UNIVERSITY,

CHENNAI, TAMILNADU.

CERTIFICATE

This is to certify that the dissertation entitled **"CLINICOMYCOLOGICAL STUDY OF VULVOVAGINAL CANDIDIASIS"** submitted by **Dr. SEENIAMMAL.S** to the Tamilnadu Dr. M.G.R Medical University, Chennai, is an original work done in the Department of Dermato-venereo-leprology, Tirunelveli Medical College for the award of the Degree of MD Dermato-venereo-leprology under our guidance and supervision during the academic period of 2012 – 2015.

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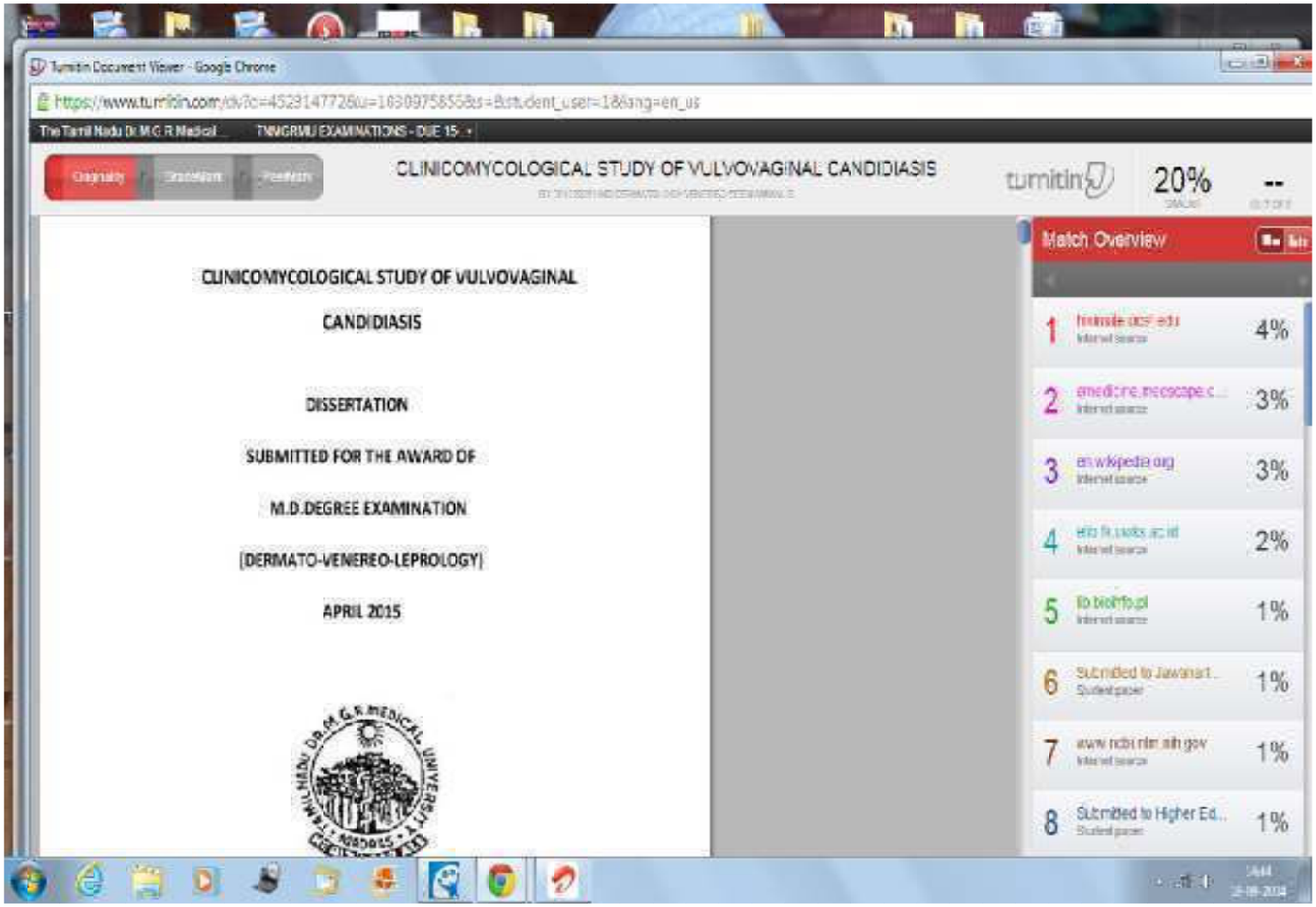
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I solemnly declared that the dissertation titled **"CLINICOMYCOLOGICAL STUDY OF VULVOVAGINAL CANDIDIASIS"** is done by me at the Department of Dermato-venereo-leprology, Tirunelveli Medical College, I also declare that this bonafide work or a part of this work was not submitted by me for any award, degree, or diploma to any other University, Board, either in India or abroad.

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CONTENTS

CLINICOMYCOLOGICAL STUDY OF VULVOVAGINAL CANDIDIASIS

Introduction

Vaginal Candidiasis is a most common fungal infection of the vulva and vagina involving nearly 75% of adult women during their lifetime. Vaginal Candidiasis is otherwise called as vaginal thrush. It is caused by Candida species. Candidiasis of vulva and vagina is found to be the second most common cause of vaginitis after bacterial vaginosis. Candida albicans constitutes 85% to 90% of cases.

Candida is a normal inhabitant of healthy mucosal surface of oral cavity, gastrointestinal tract and vagina. These are true opportunistic pathogens which utilise the host's debilitated status and gain access to the circulation and deeper tissues. However, some factors or conditions like prolonged antibiotics usage which alter the balance of micro organisms in the vaginal milieu, pregnancy, diabetes in which increased glycogen provide nutrition to the candidial growth, immunosuppressive drugs and HIV infection may result in an overgrowth of Candida. Very young and very old are also at risk of getting the infection.

The genus Candida is comprised of more than 200 species. The medically significant *Candida* species include Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida parapsilosis, Candida lusitaniae, Candida kefyr, Candida stellatoidea, Candida guilliermondii, and Candida dubliniensis. Candida albicans is the most common opportunistic fungal infection which cause a range of mild superficial infections to life threatening systemic candidiasis which occurs in immunocompromised patients. In recent years non Candida albicans species which are resistant to traditional antifungals are emerging. Nowadays candidiasis has become more and more severe due to the spread of HIV infection and the frequent use of antibiotics and immunosuppressive therapies. Furthermore, the treatment of candidiasis is difficult due to the limited choices of antifungals.

Classical clinical symptoms of vulvovaginal candidiasis include curdy white vaginal discharge and vaginal itching. Making a diagnosis of vaginal candidiasis includes performing a complete evaluation , detailed history and and physical examination, together with pelvic examination. This consists of taking a swab from the vaginal secretions and visualising it under a microscope to confirm an overgrowth of yeast. Sometimes patients may get dysuria and dysparunia. The surrounding tissue around the vulva and the vagina may become red and swollen.

Vulvovaginal candidiasis can be managed with a variety of antifungal agents, few of which are available over-the-counter. Although the way to clearly diagnose vulvovaginal candidiasis is to see the pseudohyphae under the microscope, many women take medicines themselves based on their symptoms which favours infection with drug resistant species. Proper treatment usually results in complete resolution . So in view of these factors speciation and drug sensitivity of vulvovaginal candidiasis was done and the results were analysed .

VULVOVAGINAL CANDIDIASIS

AIMS AND OBJECTIVES

This study was done in adult females in the Department of Dermatovenereology at Tirunelveli medical college with the following objectives

- To diagnose vulvovaginal candidiasis amongst female patients with genital pruritus and discharge pervaginum by clinical and laboratory methods including wet mount, 10%KOH mount and Gram's staining.
- To identify the culture characteristics and species prevalence of Candida species.
- To study the drug sensitivity pattern of vulvovaginal candidiasis those who were proved to have canidida infection by vaginal smear study.

MATERIALS AND METHODS

Our study is a open prospective study which was conducted in the Department of Dermato-Venereoleprology after obtaining clearance from Ethical committee of Tirunelveli Medical College with the co-operation of Department of microbiology, over a period of one and half years from January 2013 to July 2014.

INCLUSION CRITERIA:

1. All female patients aged more than 18 years with complaints of genital pruritus and discharge pervaginum attending the STD clinic of Department of Dermatovenereoleprology
2. Those cases proved to have candida infection by vaginal smear for wet mount, KOH and Gram's stain in the laboratory.

EXCLUSION CRITERIA:

1. Female patients aged less than 18 years.
2. Patients already treated with antifungals.
3. Vaginal smear negative for Candida.
4. Pregnant women.

Female patients aged more than 18 years with genital pruritus and discharge pervaginum were included in the study. Oral and written consent in their local language was obtained before starting the study including consent for photographs. A detailed clinical history regarding the nature of discharge, duration of illness, recurrence, contact history, associated medical disorders like diabetes, drug history and contraceptive usage was taken.

A thorough genital examination regarding sites involved, nature of the discharge and surrounding skin was done. Speculam examination was also done by keeping the patient in lithotomy position and self retaining bivalved Cusco's speculam was inserted carefully in to the vagina to view the nature of vaginal discharge, presence of erosions and ulcers. Speculum examination was avoided in unmarried female patients.

Vaginal discharge was smeared in three separate slides. Wet mount, KOH mount, gram staining were done. The patients whose vaginal smear showed Candidial pseudohyphae as elongated structure with cell wall constrictions in KOH mount and Gram positive rods in Gram's staining were chosen for study of culture, species identification and drug sensitivity. The vaginal swabs were inoculated in Sabouraud's dextrose agar and incubated at 37 °C for 48 hours. The candidial isolates were inoculated in the Chrome agar and various species were identified based on the pigmentation. All the

isolates were subjected for antifungal susceptibility testing for fluconazole, itraconazole, voriconazole, clotrimazole, nystatin, miconazole and ketoconazole by disc diffusion method. The basic investigations like blood sugar, urea, creatinine, urine routine and complete hemogram were done to look for precipitating factors. The cases were treated with antifungals according to the sensitivity and followed up after first and second week.

REVIEW OF LITERATURE

Introduction

Vulvovaginal candidiasis is defined as infection of the vulva and vaginal mucosa caused by species of genus Candida. Candidial species are able to cause superficial lesions in both oral and vaginal mucosa when the equilibrium between fungi and the host change in favour of the fungus[1]. The infection may be acute or chronic, superficial or deep and it has wide clinical manifestations. Most commonly it occurs secondary to a immunocompromised state.[2]

Prevalence

Vulvovaginal candidiasis(VVC) is the second most common cause of vaginal infections next to bacterial vaginosis in Asia and U.S. This is the first common cause of vaginitis in Europe. It is estimated that 75% of women during the fertile period have at least one episode of vaginal Candidiasis.[3] Point prevalence studies indicate that the Candida species are found in 10-50% of healthy asymptomatic females of reproductive age group (sobel et al). Hermen Gardner said 'Vaginitis cause more inconvenience than others'. About 85-90% of VVC is caused by Candida albicans.[4] Other species are C.glabrata, C.parapsilosis, C.krusei, C.tropicalis C.guilliermondii. A study in U.S isolated candida from vaginal by PCR

method in 1316 women showed Candida albicans 80.2%, Candida glabrata-14.3 %, Candida parapsilosis-5.9% , Candida tropicalis-8.0% and least percent of Candida krusei.[5]

History:

The first description for Candida infection is found in Hippocrates' "Epidemics' from 4th century BC. Rosen von Rosenstein identified Candidial infection in paediatric patients and framed description in modern medicine. In 1844 , the fungus was isolated from the sputum of a patient with tuberculosis by Bennett. It was isolated from vaginal infections by Wilkinson in 1849.Later it was isolated from blood and brain by others. It was named as 'Oidium albicans' in 1853 by Robin. `Oidium albicans` was derived from the word Candida which describes the *white Robe* (*Toga*) worn by Roman senators. Zopf in 1890 named it as Monilia albicans[6].

In 1923 Berkhout structured candida genus. Because of its genus name as monilia,it is also called moniliasis. In 1978 Torulopsis glabrata was renamed as Candida glabrata. This name was internationally accepted in 1954. The term candidiosis was used in U.K, France and Canada. Candidiasis is American English. The French word for oral thrush is Le-Muguet which means' lily of the valley.[7]

Anatomy of female genital tract:

Female genital system includes vulva, vagina, cervix, uterus tubes and ovaries. Vulva includes external genitalia and perineum. External genitalia includes mons pubis , labia majora, labia minora, vestibule, clitoris and vagina. With elevated levels of either endogenous or exogenous estrogen, the vaginal epithelium thickens due to glycogen storage. With decreasing levels of estrogen, the lining become thin and atrophic.[8]

Labia majora:

It is a fold of skin which encloses fat. Histologically, labia majora is covered with squamous epithelium consisting of sebaceous glands, apocrine sweat glands and hair follicles.

Labia minora:

It is a thin fold of skin situated inner to the labium majora. It contains veins and elastic tissues and devoid of sebaceous glands and hair follicles.

Clitoris:

It is a rounded subcutaneous structure situated at the junction of two labia minora and attached to the under surface of the pubic symphysis by suspensary ligament. It has well supplied nerve endings and is highly sensitive.

Vestibule :

It is a space situated between the anterior and inner aspect of labia minora. External urinary meatus lies just below the clitoris. Vaginal orifice lies below the meatal opening.

Vagina:

Vagina extends from cervix to hymen. Its anterior wall is 9cm in length and posterior wall is 11.5 cms. Vaginal portion of the cervix projects in to the upper portion of vagina forming anterior, posterior and two lateral fornices. Histologically it is lined by stratified squamous epithelium .Vagina is devoid of any glands. So the secretions from the vagina is derived from the transudation of epithelial cells.

Structure of vaginal epithelium:

The vaginal squamous epithelium is divided in to three layers. They are Superficial, middle and deep layers.

- ✓ Superficial cell layer have precornified cells and fully cornified cells.
- ✓ Precornified cells are large hexagonal wafer shaped cells with faintly basophoilic cytoplasm and small pyknotic nucleus.

- ✓ Cornified cells are same like precornified cells but the cytoplasm is eosinophilic .
- ✓ Middle layer consists of large ellipsoidal cells which are intermediate between basal and cornified layer.
- ✓ Deep layer cells are of two types. Basal cells and parabasal cells. These cells are small deep basophilic cells with large central nucleus.
- ✓ These cells are under the influence of hormones like oestrogen and progesterone. Vagina of female newborn is cornified because of high estrogen content obtained from maternal estrogen. After 1-2 weeks of birth the vaginal epithelium becomes thinner and remains in the same state up to puberty.
- ✓ During early proliferative phase, the vaginal squamous cells are maturing and cornification index which is the percentage of cornified cells rises. In late proliferative phase, the vaginal cells are fully mature and the cornification index is highest. During the secretory phase, the squamous cells are folded and become crumbled. Cornification index also falls.
- ✓ During pregnancy, the cornification index is low in the first half of pregnancy and further reduction occurs in the later months of pregnancy.In the post menopausal phase, the vaginal epithelium becomes thinner and parchment like.

Physiology of normal vaginal secretions:

In the reproductive age group, normal vaginal discharge consists of approximately 1-4 ml in 24 hours, which is white, transparent and odourless. It is more noticeable during pregnancy, at the time of ovulation and with oral contraceptive pills usage.

Normal vaginal discharge is formed by

- ✓ Transudation from vaginal epithelium and desquamated cells of the cornified layer.
- ✓ Sweat and sebaceous gland secretions of vulva.
- ✓ Bartholin's gland secretions
- ✓ Mucous secretions from endocervical glands.

Vaginal acidity:

A healthy adult women of child bearing age harbours lactobacillus, also called Doderllein's bacilli which are Gram positive bacilli and sugar fermenting.[9] It converts the vaginal glycogen in to lactic acid accounting for the acidic vaginal pH.

- Newborn – 5.7
- Children- 6 to 8
- Reproductive age group, pregnancy - 3.8 to 4.5
- Menopause-7

Epidemiology:

- Agent factors

Candida is a yeast like fungus. Fungi are eukayotic organisms which possess rigid cell wall made up of chitin and it contains ergosterol. Chitin is a polysaccharide consisting of long chains of N acetyl glucosamine. In addition to chitin the cell wall contains mannans which are the principal antigen component, betaglucans, and other polysaccharides. Basic differences in the membrane sterols is the target for the selective action of antifungals. Candida species are unicellular organisms that are round to oval in shape and size ranges from 2 to 6micrometer. They reproduce by asexual methods to form budding blastoconidia and sexual methods by producing ascospores and basidiospores. Then the budding forms elongate to form pseudohyphae which resemble links of sausage. *Pseudohyphae* are elongated buds with cell wall constrictions. Cells that are elongated and ellipsoidal attached side by side to one another are referred to as psuedohyphae. True hyphae are characterized by cells in cylindrical shape and are separated by perpendicular septal walls. The hyphal forms consists of conjoined cells that are divided by septal walls.

Candida species are found in animals, human and many food stuffs especially in packaged fruit juices. In humans Candida species is commonly

found in GIT, oral cavity, genital area as commensals. So infection may be caused by endogenous invasion or acquired from external sources. Asymptomatic vaginal carriage is seen in 21-32% of healthy women. One recent study analysed a group of females and frequently screened them over one year. No colonisation was observed in 30%, 70% were colonised at least one occasion, persistent colonisation was found in 4%. Higher rates are found in pregnant women associated with high Lactobacillus colonisation, diabetes melitus and post antibiotic treatment.[10] Candidiasis is the most common opportunistic fungal infection encountered in immunosuppressed patients (spinillo et al).[11] Recent studies demonstrated the ability of candida to become resistant for common antifungals.

Nomenclature of candida

Order : Cryptococcales

Class : Blastomyces

Genus : Candida

Species

- Candida albicans,
- Candida glabrata
- Candida krusei

- Candida tropicalis
- Candida parapsilosis
- Candida guiliermondii
- Candida kefyr/Candida stelloidea
- Candida rugosa
- Candida dubliniensis
- Candida vishwanathii

Candida lusitaniae

About 200 species of Candida are discovered so far of which Candida albicans is the most common organism. Only less than 10 non Candida albicans species are associated with infection. These species can be differentiated by colony characteristics, microscopy, biochemical reactions such as fermentation &sugar assimilation and growth in different media.[12]

Candida albicans:

There are 2 serotypes of C. albicans, type A and B based on their differences between mannan components of cell wall. Type A is antigenic similarities to C.tropicalis. Type-B C.albicans is commonly found in immunocompromised persons in Europe. Type A, B were common in US. Type A is also found in blood culture isolates.[13] Other Candida species apart

from C.albicans which were previously thought to be non pathogenic are now emerging as agents of infection.

C. glabrata infection is commonly seen in adults and elderly than children. C.tropicalis is most commonly encountered in patients with hematological malignancies especially in conditions with neutropenia.[14] Mouse models and some human studies showed C.tropicalis in tissues surrounded by necrosis which indicates the organism can invade the tissue efficiently. This phenomenon is due to the expression of aspartyl proteases and acid proteinase which are the virulence factors found in Candida organisms.[15] C.krusei is associated with resistance to azole antifungals. It has ability to grow in vitamin free media. C.parapsilosis is the primary cause of fungemia in neonatal intensive care units and in blood cultures due to its selective growth in hyperalimentation solution and also its ability to grow in intravenous catheter.

Virulence factors of Candida species:

- **Adhesin:**

They are the fungal surface molecules which adhere to the host buccal, vaginal, dermal epithelial cells, endothelial cells, oral and GI tissues and some inert substances such as indwelling medical catheters. Several adhesins are discovered so far. They are mannans and chitin.[16] Mannans

are mannoproteins which are proteinaceous substances. Different Candida species have different adhesins.

Biofilm formation:

After initial adherence, the fungi grow in multiple colonies forming biofilm. Biofilm is composed of proteins and carbohydrates which are responsible for the poorer response to antifungal drugs and also difficulty in clearance by host immune system. It can be demonstrated in all Candida species especially C.albicans. Biofilm formation on catheters and prosthetic valves can cause systemic disease.[17]

- ✓ Enzymes with hydrolytic activities in Candida species play major role in pathogenesis. They are aspartyl proteinases, serineproteinases, proteases, lipases, phospholipases, esterases and phosphatases. Hyphal tip with phospholipases exhibit greater invasiveness.
- ✓ Toxins which are glycoprotein extracts of Candida cell walls are lethal, pyrogenic and induce anaphylactic shock.
- ✓ Complement receptors-Candida albicans possess C3b which binds to complement as well as fibrinogen there by affecting the binding of neutrophils which in turn influence phagocytosis.
- ✓ Phenotype- switching :

It is the ability of candida to switch reversibly to different colony phenotypes ranging from unicellular budding yeast to pseudo hyphae and true hyphae. The colony morphology changes from smooth to rough and fuzzy. This phenomenon is first described by Slutsky et al in 1985, 1987. This is analogous to phase variation in bacteria. It expresses fungal plasticity and adapt the organism to various anatomical sites in human body. This property helps the organisms to evade the host's defense system.

Host factors:

<u>Predisposing factors</u>:-

1. Pregnancy have 30-40% of high risk especially in third trimester.[18,] Certain changes in the levels of female sex hormones, such as excess estrogen, make a woman more likely to develop a yeast infection. Recurrent infections are also more likely. There is no clear evidence that treatment of asymptomatic candidal vulvovaginitis reduces the risk of pretem delivery in pregnancy. Furthermore, the acidic nature of the pregnant vagina suppress the growth of other microorganisms that are naturally inhibitory to Candida. Although the high pH of 6-7 augments the initial attachment of the organism to the vaginal epithelia, the formation of germ tubes and the development of mycelia are favoured by acidic vaginal ph of less than 5.

2. Diabetes mellitus is one of the predisposing factor for recurrent vulvovaginal candidiasis. Elevated levels of blood glucose enhances the ability of C. albicans to bind to vaginal epithelial cells. Diabetes mellitus is believed to increase the propensity by raising tissue glucose, decreased phagocytosis, and altered yeast adhesion.[19]
3. High estrogen content in oral contraceptive pills (>50µg) increases glycogen content in vaginal epithelial cells which acts as good source of carbon needed for Candida for its growth and germination.[20] This hormone also accelerates the formation pseudohyphae. Progesterone, lactation are safe factors.
4. **Drugs:**
    - Frequent antibiotics clears the normal protective vaginal flora there by facilitating Candidial colonisation. The possibility of infection increases with the total duration of antibiotic use, but not related to a specific group of antibiotic.[21]
    - Systemic steroids increase the susceptibility to candidiasis by diminishing the immune function. A study done at tertiary care centre showed 19.83% association between corticosteroids and vulvovaginal candidiasis.

4 Tamoxifen usage in post menopausal women also enhances candidial infection

5. IUCD, diaphragm, spermicide, douching, perfumes and use of feminine hygienic products will produce minor trauma to the vulva and alter the vaginal bacterial flora. Douching is also a risk factor in females with recurrent vulvovaginal candidiasis caused by Candida glabrata.[22]

6. Endocrine diseases like, hypothyroidism, hypoparathyroidism, Cushing syndrome and polyendocrinopathy are associated with increased susceptibility to infection.

7. Immunosuppressive states like HIV infection, neutopenia, organ transplantation and congenital immunodeficiencies express defective killing by neutophils and macrophages thereby increasing the susceptibility to infection

8. Occlusion and maceration of skin due to tight dressings:

Perspiration associated with poorly ventilated underwear or tightly fitted clothes increases moisture and local temperature. Mechanical irritation of the genital area by clothing or sexual intercourse may also predispose to infection.

9. Nutritional factors:-

Nutritional deficiencies may alter host defense mechanisms or epithelial barrier integrity, allowing increased adherence or penetration.

- Iron deficiency causes oral candidiasis but unbound Iron enhances C. albicans growth. Vitamin A deficiency affects keratinisation. Vitamins B complex, vitamin C and folic acid are associated with higher rate of infection.
- Zinc deficiency causes chronic GI disease which can lead to recurrent vulvovaginal candidiasis.
- Protein deficiency results in impaired host defense.

Genetic predisposition:

Women those who are non secretors of Lewis antigen, a glycoprotein that inhibit the binding of Candida to the vaginal mucosa are vulnerable to recurrent vulvovaginal candidiasis.[23] Genetic polymorphisms , inherent hypersensitivity to yeast are also risk factors.[24,25] In healthy women, the vaginal epithelial cells show some anti Candida activity which is low in patients with recurrent vulvovaginal candidiasis.[26]

Atopy

Women with recurrent vulvovaginal candidiasis(RVVC) were found to be associated with atopy in some instances. A study on ninety five patients with RVVC were compared with 100 controls, it showed 74% incidence of allergic rhinitis against only 42% in control group.[27]

Few case reports showed high titres of eosinophils and *Candida* specific IgE in vaginal washes indicating that a local immune response may play some role in RVVC.[28,29] However, the patients with RVVC with evidence of IgE or eosinophils in vaginal washes is much lower than the healthy females.[30,31]

10. Sexual factors:

Orogenital sex may facilitate the introduction of microorganisms.[32] The role of sexual transmission is controversial, even if some male sexual partners experience a transient erythema, itching and burning sensation in the genitals few minutes to hours following sexual intercourse. A randomized controlled trial evaluated the outcome of treating male sexual partners with ketoconazole had similar recurrence rates as that of the untreated partner groups at six months and one year.

Serum factors*:*

Substances in the serum such as transferrin and clumping factor enhances germ tube and mycelia formation thereby facilitating invasion.[33] These factors also cause inhibition of T cell function which can be reversed by antifungals

HIV and vaginal candidiasis:

Acquired immunodeficiency syndrome (AIDS) is a chronic disease primarily affecting the human immune system caused by the human immunodeficiency virus (HIV). It is a two single stranded RNA virus which gradually reduces the normal functioning of the immune system and thereby, making the individuals vulnerable to infections and other diseases. The virus initially destroys CD4 T-lymphocytes which helps to organize the immune system's response to infection and disease. The CD4 cell count is considered as a marker to determine the progression of the disease. Patients with a CD4 cell level less than 200 cells per microlitre have progressed to AIDS. Another name for CD 4 cells is T-helper cells. CD4 cells are made in the lymph nodes , spleen, and thymus. In addition to other tests, the CD4 count is necessary for staging HIV disease, to predict the progression and guidance to treatment. Complications of HIV are less with higher CD4 count and life expectancy is more.

Vulvovaginal candidiasis (VVC) is one of the most common fungal infection which recurs frequently in females with human immunodeficiency virus (HIV) infection. Inspite of only rare chances of systemic fungal infection and mortality, it is essential to prevent and treat candidial vulvovaginitis for improving the quality of life of HIV positive patients. Oral, esophageal and vulvovaginal candidiasis are the common forms of

Candida infection in HIV positive individuals. They are the indicators of severity of immune deficiency and forerunners of systemic opportunistic infections. Prevalence of vaginal Candidiasis is directly propotional to the immunodeficiency in HIV positive individuals . Immuno suppression and frequent usage of antibiotics for various bacterial opportunistic infections are the reasons behind this. A cross-sectional study of 833 HIV positive and 427 HIV negative women , the yearly incidence of vaginal candidiasis was similar (9%) in the 2 groups. A study on 205 HIV positive women, the risk of developing symptomatic VVC is increased 5-6 times for those with CD4 count of <200 cells/microliter. The rate of candidial colonization of vagina in immunocompetent (CD4 cells>500) HIV positive women and HIV negative women was the same,[34] although the relationship between HIV infection and vulvovaginal candidiasis remains uncertain. There is no much difference in the symptomatology in HIV positive and negative women.(Spinillo et al). Symptomatic vulvovaginal candidiasis is more common in HIV positive patients and directly proportional to the severity of immunosuppression. Symptoms of vulvovaginal candidiasis generally get worsened during menstruation because the hormonal changes make the vaginal mucosa suitable for fungal growth. In addition, in HIV positive women, chronic exposure to systemic azole antifungals results in growth of non Candida albicans species in the the vagina.

The individual Candida species affecting patients with HIV infection are not different from those in other immunosuppressed hosts.[35] C dubliniensis is frequently identified in HIV positive persons, though it is presently impossible to differentiate from C. albicans in its clinical presentation.[36,37] .There are no apparent differences in the virulence of organisms isolated from HIV positive or HIV negative persons. Recurrence can result from the same or from different species of Candida.[38] Reinfection with different species of Candida is more common in persons with previous antifungal therapy and low CD4 lymphocyte counts. Candida dubiliniensis is commonly occurring in HIV positives. A study conducted by Deepa babin et al revealed 1.6% of vaginal candidiasis belongs to C.dubliniensis.

The development of mucocutaneous candidiasis depends on many factors. The degree of immunosuppression is the vital factor. Other host factors responsible for the protection against Candida infections include blood group secretor level such as presence or absence of specific Lewis antigens, rate of salivary flow, antimicrobial activity of saliva, the epithelial barrier integrity, presence of normal bacterial flora, and the local immunity.[39] Several studies suggest an impaired host immune response to Candida in persons with HIV infection. Plasma HIV-1 RNA levels are found to be elevated in patients with higher incidence of mucocutaneous candidiasis and colonization with Candida.[40]

Refractory candidiasis:

Refractory vaginal candidiasis is fairly less common. It is defined as the failure of response to antifungal drugs for a standard time duration that is for 14 days with suitable doses.[41] Anti fungal resistance is classified as clinical and invitro resistance. Clinical resistance is due to non compliance with the drug regimen that leads to low serum and tissue levels of the drug. This is most common in HIV positive patients due to profound immunosuppression where a high dose antifungals are unable to eliminate the fungus.

Invitro resistance is subdivided in to primary and secondary. In primary resistance the organism is naturally resistant to antifungals. Eg. C.krusei to fluconazole . In secondary or acquired resistance the organism becomes resistant during the course of the treatment. This type is more common in HIV positive individuals. Fluconazole resistant cases are viewed with particular attention due to considerable morbidity and often requires alternate parenteral antifungals for the treatment. Refractory candidiasis usually occurs in patients with advanced HIV disease with CD4 counts less than 50 cells/μL who were already treated with antifungal therapy for prolonged period.[42]

Refractory candidiasis is hard to treat and gradually become unresponsive to therapy . The most significant step is to decide what medications and dosages have been tried and whether the patient's adherence to therapy is sufficient. Removal of any interacting drugs or increasing the drug dosage of the antifungals may cure the disease in some persons. Amphotericin B, itraconazole, or flucytosine are the alternative therapeutic strategies. Other options for the treatment of fluconazole-resistant isolates include voriconazole, micafungin, caspofungin, and anidulafungin. Antiretroviral therapy must be optimized in patients with refractory candidiasis. Treatment with protease inhibitors is found to be useful in the clinical improvement in difficult-to-treat cases.[43] Protease inhibitors have been shown to inhibit *Candida* secretory aspartic proteases, indicative of direct antifungal activity against *Candida*. The duration of antifungal treatment for refractory disease depends on the response, but in general a course of 14 days is given for vaginal disease. Higher relapse rates seen in persons with refractory disease, and suppressive therapy is commonly required. In difficult cases, weekly therapy of 2-3 times a week or daily suppressive therapy may be required to avoid relapse.

The incidence of systemic candidiasis in AIDS is probably <1%. Patients who develop systemic candidiasis are known to have risk factors like, chemotherapy, neutropenia, indwelling intravenous catheter and parenteral alimentation.[44] The occurrence of invasive candidiasis in HIV

patients is rare. The pathogenesis of invasive candidiasis involves discontinuity of a mucosal surface barrier with subsequent haematogenous dissemination secondary to macrophage and neutrophil dysfunction in conditions that are not distinctive feature of HIV disease.

In a published study of 14 cases of Candida meningitis in HIV positive patients, a history of at least one predisposing factor was noted in ten of the patients, nine of them were intravenous drug abusers. Of interest, 4 patients had no risk factors. The HIV positive patients with Candida meningitis can be treated with prolonged suppressive therapy with fluconazole with a regimen similar to that used in the treatment of cryptococcal meningitis.

HAART is the only best method of reducing the incidence of mucocutaneous candidiasis. Most studies demonstrated, the use of HAART reduces the rate of colonization and severity of clinical manifestations.[45] This is correlated with reduction in HIV-1 RNA levels in the plasma. The other effective interventions include smoking cessation, the treatment of opportunistic infections, good hygiene, avoidance of unwanted antibiotics, steroids, and antifungal drugs. So the most important way of preventing mucocutaneous candidiasis is improvement of immunodeficiency associated with HIV infection .

Immunology:

Candida species are commensal as well as opportunistic pathogens of mucosal tissues. Both innate and acquired immunity play a vital role in keeping the organism in the commensal state and preventing it to enter in to the systemic circulation. Neutrophils plays important role in the protection against systemic infections, whereas cell-mediated immunity (CMI) by Th1-CD4+ T-lymphocytes aids protection against mucosal infections. Candida-specific antibodies are controversial in the protection against infection. Some studies done on mucosal innate resistance recently established epithelial cells from vaginal lavages and saliva of healthy individuals inhibit the growth of Candida in vitro. Oral epithelial cells obtained from HIV positive candidates suffering from oral candidiasis have significantly reduced activity. So these studies indicate some protective role for the epithelial cells and suggesting that immunity to Candida is site-specific and compartmentalized.

The level of innate immunity to pathogenic fungi is high in most humans as evidenced by the fact that the fungal infections are usually mild and self limiting. The intact skin and mucosal surfaces are the primary barriers to infection. Epithelial cell turn over, fatty acid content of the skin, low ph of vagina are the important factors of host resistance. Bacterial flora of the skin and mucosae compete with fungi and inhibit their unhindered proliferation. Alteration in bacterial flora and any break in the natural barrier facilitate infection.

The outcome depends on the virulence of organism and size of inoculam and adequacy of host defenses. Cell mediated immunity provides protection against pathogenic Candidial colonization. Phagocytosis by neutrophils is the primary mode of elimination of infection. Some evidences showed that antibody along with complement play a role in eliminating fungi from the body.

Candida antigens:

1. Cell wall antigens
2. Cytoplasmic antigens

These antigens are useful for serological applications i.e agglutination reactions. Mannan is the major antigenic component of cell wall. Subtle variations in the linkage and side chains of mannose residues led to the serotyping and speciation. The glucan polymers which present in more abundance is immunologically less active. The number of antigenic components in broken cells is also significant.

Pathogenesis:

Most of the candidal species produce virulence factors including protease factors. The species which do not have the virulence factors are less pathogenic. The ability of yeast forms to adhere to the host's epithelium is a crucial step in the hyphae formation and pathogenic tissue invasion. Elimination of the competing bacteria from the skin and mucosae results in

reduced nutritional and environmental competition that favours the growth of Candidal organisms.

Additional researches proved that cytokines and interleukins have a significant role in candidial infection. In keratinocytes, *C albicans* phospholipomannan provokes an inflammatory response through toll-like receptor 2. *C*andida albicans reduces the expression of interferon-gamma–inducible protein-10 in human keratinocytes. These factors explain innate defenses against candidal organisms. Interleukin 12 receptor β1 deficiency predisposes to candidiasis, most commonly of mucocutaneous candidiasis.

Steps in pathogenesis:

- Adherance to epithelial endothelial surface
- Invasion
- Host immune response

1) Adherence:

C. albicans have more affinity towards vaginal epithelial cells

Order of affinity:

C.albicans>C.tropicalis>C.parapsilosis

Factors influencing adherence:

- Local vaginal pH:

  Candida adhere to the vaginal mucosa even if the pH is acidic.

- ❖ Removal of competing bacteria leads to increase in the number of yeasts thereby facilitating invasion. In some areas like web spaces the bacteria especially gram negatives act as co pathogens rather than competitors.
- ❖ Nutrients:

  Glucose is the crucial factor in maintaining balance between Candida and bacteria in saliva . If there is high glucose as in diabetes there is only low level of competition exists between them and Candidal invasion occurs very easy.
- ❖ Hormonal level- Estrogen increases the glycogen content of the vaginal epithelial cells thereby increases the affinity.
- ❖ Presence of lg A interfere with adherence
- ❖ Cell surface hydrophobicity- germ tube formation is enhanced by hydrophobicity. Adherence results in colonization at the local site or it invades deeper tissue and cause symptomatic disease.

2) Invasion:

During active infection, yeast form is converted in to hyphal form. Phospholipases at the tip of hyphae is related to greater invasiveness compared to yeast form. Hyphae is larger than yeast. Hyphae which are longer than 200 micrometer are more resistant to phagocytosis. Invasion is aided by various enzymes like proteinases which cleave the peptide bonds. Phospholipases enhance invasion by proteolysis in keratinocytes. Aspartate

proteinases are collagenolytic. Another study revealed elevated levels of hyaluronan in patients with RVVC, which is associated with severe itching , burning and vaginal discharge.

Host immune response:

Candidial infections stimulate both Cell mediated[CMI] and humoral immune responses. But CMI is the first line of defence.CMI includes activation of neutrophils, mononuclear leucocytes, keratinocytes and dendritic cells. Candida albicans interact with the major antigen presenting cells i.e., dendritic cells. The dentritic cells stimulate Candida albicans specific lymphocyte proliferation which recognize them via mannose receptors. The glycosilated portion of Candida mannoprotien 65 (MP65) stimulates Th1 responses secreting cytokines like IL2,IL12,TNF alpha and IFN gamma the production. T cell activation via toll like receptors leads to cytokine release and inflammatory cascade proceeds.

In addition to cell mediated immunity, humoral immunity also act against Candida. Antibodies have been recovered from experimental animals with vaginitis. IgM and IgG antibodies are found in mucocutaneous candidiasis. Anti Candida IgE antibodies have been demonstrated in patients with allergy.[45] witkin et al 1989 demonstrated IgE antibodies in women with vulvovaginitis indicating a possibility of type 1 hypersensitivity in the pathogenesis of vulvovaginitis. Local immunity also plays an important

role in defense against Candida. IgA antibodies have been demonstrated in vaginal fluid.[46]

A study in 28 patients with RVVC showed T cell dysregulation with increased production of IFN ϒ in RVVC patients. This can be exacerbated by increased level of estrogen in follicular phase of menstrual cycle.[47] Some patients responds to hyposensitisation with Candida antigen given as a course of few weeks to months which exhibits the role of hypersensitivity reaction in the pathogenesis.

There is another emphasis that Th1 and Th2 response to candida cross regulate each other. If there is increase in antigen load, there may be induction of Th2 response of inhibition of normal protection aided by Th1 activity.

Histopathology :

- H&E staining shows spongiotic and subcorneal pustular dermatosis like picture with mixed dermal infiltrate.
- Fungal organisms are demonstrated within stratum corneum by special stains like Gomori methenamine silver stains. Spores appear as 3-6 micrometer round to ovoid in shape. Pseudohyphae are 2-4 um, septate, branching at right angles. Constrictions are present at the site of septations.

Clinical features:

Clinical features ranging from asymptomatic to severe vulvovaginal candidiasis.

Clinical classification:

Centers for disease control and prevention classified VVC in to two groups.[48]

Uncomplicated VVC:

- Single episode or less than 4 episodes in a year.
- Mild or moderate symptoms
- Causative organism is *Candida albicans* .

Complicated VVC:

- Recurrent episodes- 4 or more episodes per year
- Severe symptoms of vulvovaginitis
- Associated with pregnancy, poorly controlled diabetes and HIV infection
- Caused by the non *Candida albicans* species

Acute Vulvovaginal Candidiasis:

Symptoms

- Vaginal pruritus
- Soreness/Burning pain
- Vaginal discharge

- Dyspareunia
- Dysuria as the urine come in contact with the inflammed vulval and vaginal mucosa

Signs:

- Erythema,
- Fissuring
- Edema of vestibule , labia
- Thick white curd like or 'cottage cheese like' vaginal discharge adherent to the walls of vagina, labia which bleeds on removal.
- Maceration in the adjacent skin
- Satellite pustules in the perineum, groins and thighs.

Chronic VVC:

Symptoms:

- Severe pruritus
- Burning sensation
- Irritation
- Pain

Signs:-

- Lichenification of Vulva
- Greyish sheen made up of epithelial cells and organism cover the area.

Differential diagnosis:

Infectious conditions:

- Recurrent bacterial vaginosis:

  It presents as profuse greyish white thin homogenous discharge lining the vagina and smear positive for clue cells. Vulval irritation is mild. Episodes are not related to menstruation.

- Trichomonas vaginalis infection:

  Trichomonas vaginalis is distinguished from VVC by the presence of copious greenish frothy discharge with fishy odour associated with intense pruritus. Wet mount of vaginal smear show motile Trichomonads. Episodes occurs most commonly in the post menstrual period.

- Chlamydia infection is distinguished by the presence of cervical erosion, discharge, fornicial tenderness.

- Recurrent genital herpes present with either vesiculation or painful superficial ulcers with polycyclic borders. Tzanck smear show multinucleated giant cells.

- Genital scabies: nocturnal pruritus, family h/o itching, excoriated papules over the genital region, around the umbilicus ,medial thighs and around the areola.

Non infections conditions:

- Allergic vulvitis
- Chemical vulvitis
- Contact dermatitis:

The diagnosis is based on the patient's detailed history and physical examination. Pruritus is the general symptom. An acute reaction may develop as a result of exposure to a potent irritant that involves the mucosa, leading to burning sensation, pain followed by oozing, which may lead to secondary infections.

- Atrophic vaginitis-

  Women with mild to moderate vaginal atrophy (60-90%) may notice a slight decrease in vaginal lubrication. Vaginal dryness, dyspareunia, Postcoital burning, leucorrhea and occasional spotting are other features. The vagina appears thin, with occasional petechiae and diffuse erythema and with reduced vaginal rugosity. pH is 5-7. A serosanguineous discharge may be present. A wet mount often shows white blood cells and a paucity of Lactobacillus.

- Idiopathic vestibulitis syndrome
- Desquamative inflammatory vaginitis
- Erosive lichen planus
- Dermatosis like Eczema, Atopy and psoriasis
- Physiological leucorrhea:

It is a natural discharge secreted by the vaginal epithelia due to some hormonal and metabolic, changes in the body itself. This is normally observed in females of child-bearing age group. The discharge is normally colourless, sticky, not offensive or foul smelling.. It may not normally stain the clothing or just dampen. There might be a feeling of weakness and fatigue but there is no burning, itching and erythema.

Laboratory diagnosis:

The diagnosis of VVC is often made clinically and treatment can be started empirically. In clinically doubtful cases we opt for 10%KOH mount and Gram's staining.

- Vaginal pH- vaginal swab should be taken from lateral vaginal wall & placed on pH paper. In Candidiasis, the pH is between 3 and 4.5. Contamination with blood, cervical mucus, semen and local medication interfere with interpretation of results.

Direct microscopy

Direct microscopical examination of clinical specimens reveals budding yeast cells called blastoconidia,2-4 μm in diameter with pseudohyphae showing regular points of constrictions resembling links of sausage.True septate hyphae may also be produced by C.albicans and C. dubilinensis.

❖ **Wet mount-saline preparation:**

A sample of vaginal secretion is smeared on a glass slide, a drop of saline is added, coverslip applied over it and seen under microscopy for presence of pseudo hyphae. It can detect 40-60% of symptomatic cases.

❖ 10%KOH mount-vaginal smear is made on a glass slide and one drop of 10% KOH is added. Apply coverslip over the smear. Gentle warming of the slide or addition of dimethyl sulfoxide causes rapid clearance of the keratin and other cellular debris, leaving the fungal element intact. Microscopic visualization reveals budding yeasts and pseudohyphae. It has 70% sensitivity.

❖ Calcofluor white stain fluoresces under filtered UV light as bright green because of its binding to polysaccharide in the chitin of the fungal cell wall.

❖ Gram stain- Thin vaginal smear is made. Methyl violet is added and washed after I minute. Then Gram's iodine is added to retain the primary color. Then acetone or alcohol is added to decolorise for few minutes. Finally carbol fuschin 1:20 dilution is added as a counter stain. After washing and drying it is seen under microscopy. Yeasts and pseudo hyphae appear as Gram positive elements. It has 65-68% sensitivity.

❖ Vaginal swab smear stained by methyleneblue & examined microscopically shows presence of regular blastospores as well as pseudo hyphae.

- H&E Stain and Gomori's methenamine silver stains demonstrate fungal elements in tissues.

Germ tube test :-

This test proves yeast germination. Suspected colonies are incubated in sheep or normal human serum at 37°c for 2-3 hours. A drop of suspension is spread on a slide and examined under microscope. Germ tubes are seen as long tube like projections extending from the yeast cells. There is no constriction at the point of attachment of pseudohyphae. This test is known as REYNOLDS BRAUDE phenomenon.

Chlamydospore formation:

Yeast produces chlamydospores on cornmeal polysorbate 80 Agar at 22°c to 25°c in 48-72 hrs. Chlamydospores are refractile thick walled cells produced under nutrition poor, oxygen deprived conditions at low temperatures.[49]

Yeast assimilation test :

It represents safest identification of non-candida albicans species based on the ability to assimilate coal hides (organic compounds). Agar plates with Candida on which paper discs impregnated with different carbohydrates are placed. Candidial growth around a particular disc is an indication for assimilation of that particular carbohydrate.

Fermentation reactions:

It is done in Liquid media supplemented with different carbohydrates and colour indicator. Durhams tube is used to indicate gas production in the fermentation reaction.[50] Acid production and pH changes are indicated by various colour changes.

Culture:

Sabouraud's dextrose agar [SDA] otherwise called Nickerson's medium is the most common medium used for cultivation of fungi, moulds and yeasts. It contains glucose, neopeptone, poly peptone agar and supplied with chlorheximide, chloramphenicol and gentamycin to prevent bacterial contamination. Glucose provides carbon and peptone agar provides nitrogen. The pH of the media is slightly acidic[5.6].The colonial and microscopical morphology is of little clinical importance. Vaginal swabs are inoculated in SDA agar and incubated at 25°c and 37°c .The colonies appear in 3-4 days. Most of them produces smooth creamy white colonies. Some produces wrinkled dull colonies.

- Candida albicans produces cream coloured smooth pasty colonies
- Candida tropicalis produces cream coloured to white, glistening to dull, smooth or wrinkled colonies with mycelial fringe.

- Candida glabrata produces smooth cream, coloured , glistening, small colonies.
- Candida parapsilosis produces creamy colonies in lacy pattern.
- Candida kefyr produces smooth soft creamy colonies.
- Candida guillermondii produces thin flat, glossy, cream to pink glistening smooth wrinkled colonies.
- Candida krusei produces flat dull dry smooth or wrinkled colonies with lateral fringe.

HI chrome differential agar(HIMEDIA):

CHROM agar is rapid plate based test for isolation and identification of various Candida species simultaneously.[51] It contains chromogenic material which is acted upon by distinct enzymes secreted by different Candida species to yield a characteristic colony colour. It contains glucose, peptone, agar, chromogenic mix with the pH adjustment of 6.1. It should be stored in 2-8degree C away from direct light. Sample has to be inoculated and incubated at 37^{o} c for 48 hrs.

Characteristics of various Candida species:

C.albicans:

C.albicans grow as a medium sized, smooth, green to dark metallic green colored wet colonies at 48 hours.

C. krusei:

It grows as a large, flat, spreading, rough to crenated , pink coloured dry colonies.

C.tropicalis:

Medium sized smooth medium to dark metallic blue coloured colonies appear in 24 hours.

C.glabrata :

It produces medium sized pink coloured wet colonies with darker mauve center.

Other species – white to pink coloured colonies

Non culture methods:-

Differentiation of diverse species of candida in laboratories is done by time consuming unreliable methods. Discovery of PCR overcome this problem.

Serological tests:

- Antibodies to mannan proteins
- Gel immunodiffusion, immunoelectrophoresis, ELISA detect antibodies against enolase and heat shock proteins.
- Detection of Arabinitol in the body fluids of infected persons.

Polymerase chain reaction[PCR]:

Polymerase chain reaction is a method by which an area of DNA molecule is chosen and amplified. This method is very expensive but much more specific than conventional methods. It can detect <10 yeast colonies in the vaginal swab. So it is highly sensitive but not suitable for differentiating the asymptomatic candidiasis from others.[52] Candida albicans DNA can be measured by PCR for the diagnosis of invasive candidiasis.

Modifications of PCR

- Single, direct PCR
- Multiplex PCR-this test is much faster and more sensitive.
- PCR with dot blot hybridization-This method detect candida in blood culture samples targeting specific rRNA sequences.

TREATMENT

A wide variety of drugs are effective for the treatment of candidiasis. The mainstay of treatment of vulvovaginal candidiasis is the antifungals.An antifungal agent is a drug that selectively eliminates fungal pathogens from a host with minimal toxicity to the host. Besides antifungal agent clinical response is determined by the severity of disease and patient adherence to the treatment and the pharmacological properties of the drug. Treatment of vulvo vaginal candidiasis is very simple and most of the strains respond to therapy. Randomized studies show little difference between systemic and

topical therapy. Milder forms can be managed with topical therapy. Moderate and severe cases needs systemic therapy.

The discovery of antifungal agents has lagged behind that of antibacterial agents. This is due to the cellular structure of the organisms involved. Bacteria are prokaryotic and hence offer multiple structural and metabolic targets which are different from those of the human host. But fungi are eukaryotes, and consequently most agents that are toxic to fungi are also toxic to the host. Furthermore, because growth of the fungi is slow and often exhibit various cellular forms, they are more difficult to quantify than bacteria. This difficulty complicates experiments aimed to evaluate the in vitro or in vivo properties of a potential antifungal agent.

Major developments in the research of the azole antifungal agents during the 1990s gave expanded options for the treatment of many opportunistic and systemic fungal infections. Fluconazole and itraconazole have proved to be safer than both ketoconazole and amphotericin B. Inspite of these advances, systemic fungal infections remain difficult to treat, and drug resistance to the available drugs is upcoming. Use of the newer azole group of drugs in combination with other antifungal agents with different mechanisms of action is likely to provide enhanced efficacy. Second-generation triazoles developed to provide extended coverage of opportunistic and emerging fungal organisms, as well as to overcome resistance to older drugs.

Classification of antifungals:

Polyenes:

1. Nystatin
2. Amphotericin B

Azoles

1. **Imidazoles**
    - Clotrimazole
    - Ketoconazole
    - Miconazole
    - oxiconazole
2. **Triazoles**
    - Fluconazole
    - Itraconazole
    - Voriconazole
3. **Allylamines**
    - Naftifine
    - Terbinafine
4. **Benzylamines :** Butenafine

5. **Echinocandins:**
    - Capsofungin
    - Micafungin
    - Anidulafungin
6. **Hydroxypyridone:** Ciclosporin
7. **Heterocyclic benzofurans:** Griseofulvin

Polyene Antifungal Drugs

Nystatin, amphotericin, and pimaricin (Natamycin) belongs to this group. They interact with sterols in the fungal cell membrane to induce osmotic instability. Ergosterol in fungi and cholesterol in humans form channels through which small molecules leak from the inside of the fungal cell to the outside.

Amphotericin B:

It is derived from bacteria Streptomyces nodosus.it has broad spectrum of action against Candida and other deep fungal infections.it is fungicidal at high serum concentrations. It is not absorbed orally. It has poor CSF penetration.It is metabolised in the liver and excreted through both urine and bile. It enhances the antifungal action of Flucytosine.

The adverse effects are very high which include

- Acute reaction following each infusion which is characterised by fever, chills, nausea, vomiting, myalgia and dyspnoea lasting for 2-5 hours injection of Hydrocortisone 0.6 mg/kg with the infusion may reduce the intensity of reaction
- Nephrotoxicity
- Bone marrow suppression and anemia
- CNS toxicity

Azole Antifungal Drugs

Ketoconazole , fluconazole, and itraconazole belongs to this group. They inhibit cytochrome P_{450}-dependent lanosterol 14α-demethylase involved in the biosynthesis of ergosterol, which is necessary for the integrity of the cell membrane structure and function of fungus. Inhibition of this enzyme causes abnormal sterol accumulation inside the cell and leads to fungal cell death.

Allylamine and Morpholine Antifungal Drugs

Allylamines inhibit ergosterol biosynthesis at the level of squalene epoxidase. Terbinafine and naftifine are included in this group. Amorolfin, the morpholine drug, inhibits the same pathway at a later step.

Echinocandins and cyclo peptides:

They are the newly discovered class of fungicidal agents. They noncompetetively inhibit the fungal cell wall β-D-glucan synthesis via inhibition of the enzyme 1,3-β glucan synthase. Examples of echinocandins include anidulafungin, caspofungin and micafungin. Adverse events are fever, nausea, vomiting and infused-vein complications. They are less toxic drug. This drug causes embryotoxicity hence contraindicated in pregnancy. It can be used in azole resistant Candida infection.

Antimetabolite Antifungal Drugs

5-Fluorocytosine acts as an inhibitor of both DNA and RNA synthesis via the intracytoplasmic conversion of 5-fluorocytosine to 5-fluorouracil. Fluorocytosine is available in the tablet form.

Side effects:

- Nausea, vomiting, diarrhea, GI bleeding
- Renal dysfunction
- Hepatitis
- Thrombocytopenia, anemia, and leukopenia.

At the manufacturer level, maintaining flucytosine levels from 25 to 100 µg/mL is recommended. Laboratory monitoring including complete blood count, renal function test and liver function tests is mandatory.

Drug interactions:

Azole antifungals are metabolized by cytochrome P450 enzymes and have considerable interactions with the other drugs metabolized by this enzymes. Metabolism of fluconazole is fastened by enzyme inducers like rifampicin and anti epileptics. Fluconazole has low propensity to cause interaction. But other azoles like ketoconazole, itraconazole and voriconazole are contraindicated with terfenadine, astimizole,cisapride as it produces ventricular arrhythmias. These drugs also increase the blood levels of sulfonylurea, statins, ergot alkaloidsprotease inhibitors especially ritonavir when given in the full dose(400mg)

AZOLES:

Structure :

An **azole** is a class of five membered nitrogen heterocyclic ring compounds containing at least one other non-carbon atom of either nitrogen, sulfur, or oxygen. The basic compounds are aromatic and have two double bonds. There are successively reduced analogs called azolines and azolidines with fewer. Only one, lone pair of electrons from each heteroatom in the ring makes the aromatic bonding in an azole. Names of azoles maintain the prefix upon reduction (e.g., pyrazoline, pyrazolidine). The numbering of ring atoms in azoles starts with the heteroatom that is not part of a double bond, and then proceeds towards the other heteroatom.

Classification of azoles:

The azole antifungals include two classes, imidazoles and triazoles, which share the same mechanism of action and antifungal spectrum . The systemic triazoles are metabolized very slowly and have less effect on human sterol synthesis than the imidazoles. Because of these advantages, new drugs under development are mostly triazoles. Of the drugs now on the market, ketoconazole, clotrimazole, miconazole, econazole, oxiconazole, sertaconazole, butoconazole and sulconazole are imidazoles. Fluconazole, terconazole, itraconazole, , voriconazole, posaconazole, and isavuconazole which is an experimental drug are triazoles. Ketoconazole and miconazole have more effect on mammalian cytochromes than triazoles and tend to have severe adverse effects. Hepatotoxicity is common to all of them, and the risk of endocrine dysfunctions also exists,

Mechanism of action

At concentrations achieved after systemic administration, the major effect of imidazoles and triazoles on fungi is inhibition of a microsomal enzyme 14- alpha-sterol demethylase. Imidazoles and triazoles impair the biosynthesis of ergosterol for the cytoplasmic membrane and lead to the accumulation of 14-alpha-methylsterols. These intermediate metabolic products may disrupt the close packing of acyl chains of phospholipids and impair the functions of certain membrane-bound enzyme systems, thus

inhibiting growth of the fungi. Some azoles directly increase permeability of the fungal cytoplasmic membrane, but the concentrations required are only obtained with topical use.

Ketoconazole:

It is the first orally available imidazole. It is well absorbed orally. The absorption is pH dependent and variable with food.It reaches its peak plasma concentration at 1-2 hours of single dose.The drug does not penetrate CSF. So it is not used in fungal meningitis.It is metabolized in the liver by the enzyme cytochrome 3A4. It is excreted in urine and feces.

Adverse drug reactions:

Hepatotoxicity is dreadful complication especially in patients with previous liver disease. Others are nausea, vomiting, abdominal pain, head ache, giddiness, pruritus, gynecomastia, impotence ,loss of libido and oligospermia due to blockade of androgen synthesis by displacing testosterone from binding sites. Females get irregular cycles because the drug suppresses estradiol synthesis. It blocks the cortisol response to ACTH leading to adrenal insufficiency. It is contraindicated in pregnancy (categoryC)

Interactions:

- If coadministered with H2 blockers, Proton pump inhibitors and antacids, oral absorption of ketoconazole is reduced because of lowered gastric acidity.
- Rifampicin, INH and phenytoin decrease the efficacy of ketoconazole as they are cytochrome enzyme inducers.
- Ketoconazole is the Potent cytochrome enzyme inhibitor thereby increasing the level of cyclosporin, statins.
- With Terfenadine, astemizole and cisapride it causes ventricular tachycardia.

Fluconazole:

- Triazole are fungistatic. The imidazole group is substituted by nitrogen group which shows improved antifungal activity and resistance to metabolic degradation. Mechanism of action is inhibition 14α demethylase in fungal cell wall. It is supplied in IV, oral route, and eyedrops (0.3%) is also available. It has extensive absorption of >90% which is not gastric Ph dependent. The t1/2 is 25 to 30 hrs. Hepatic metabolism is less and 80% of the drug is excreted unchanged in urine. As the drug crosses blood brain barrier, it is useful for the treatment of fungal meningitis.

Adverse reactions:

- Gastro intestinal symptoms like nausea, abdominal pain pain, diarrhoea
- Torsades de pointes with/without concomitant drugs like cisapride and terfenadine
- Hepatotoxicity
- Rarely Steven Johnsons syndrome,toxic epidermal necrolysis
- Pregnancy category C

Drug Interactions:

- It is a Cytochrome P450, Cytochrome 2C9 Inhibitor in dose dependent manner. So influence the efficacy of the drugs metabolized by this enzymes.
- It precipitates arrhythmias if co administered with Cisapride, terfenadine
- Caution to be taken in patients having hypersensitivity to azoles, proarrhythmic cardiac conditions,renal impairment,Pregnancy and lactation

Itraconzole:

- It is a broad spectrum fungistatic. It is also a 14α demethylase inhibitor
- It inhibits cyclic oxidase and peroxidative enzymes leads to increased peroxide generation and degradation of fungus at sub cellular levels.
- Direct membrane damage by acting on membrane phospholipids
- Food and gastric acidity enhances the absorption. So the absorption is decreased in HIV patients due to gastric hypochlorhydria.
- Hydroxypropyl beta cyclodextrin oral solution form exhibits better absorption in empty stomach and in alkaline gastric ph.
- It undergoes hepatic metabolism via cytochrome3A4 and excreted in feces and urine[30%]. In children less than 5 years, the drug attains low serum levels, so twice daily dosing is needed. The drug after intake is distributed to skin and its appendages like nail& nail bed, sebaceous and sweat glands. It is measurable in stratum corneum 3-4 wks after discontinuation of drug. It donot penetrate CSF. The drug is indicated in candidial infection involving skin, mucosa,nail as well as deep mycosis.

Contraindications:

- Hypersensitivity reactions
- Along with cisapride, midazolam, pimozide, quinidine, doefitillide

Use with caution :

- Hepatic disease
- Renal disease,
- COPD
- Cardiac diseases[CCF,IHD,valvular HD-negative inotrpic effect]

Voriconazole:

- It is 2nd generation broad spectrum triazole active against severe fungal infections Oral candidiasis ,Chronic muco cutaneous candidiasis, resistant candida and fusarium infections

Adverse drug reactions:

- SJS,TEN, Erythema Multiforme
- Pseudoporphyria
- Discoid Lupus Erythematosus
- Photosensitivity reaction

Clotrimazole

It is available as 1-2% cream, lotion lozenges, troches, powder, spray and solution. It causes leakage of intra cellular proteins, break down of nucleic acid, potassium efflux and finally cell death.

Adverse drug reactions:

- Rashes, itching, stinging sensation and burning
- Irritant and allergic contact dermatitis especially to the ingredients and preservatives
- Urticarial rashes

Nystatin

It is a polyene antifungal derived from a bacteria, Streptomyces noursei. Currently, no injectable formulations of this drug is available because of its toxicity when the blood levels are elevated. The biosynthesis of the the compound involves enzymes like GDP-mannose dehydratase (nysIII), P450 monooxygenase (nysL and nysN), aminotransferase (nysDII), and glycosyltransferase (nysDI).

Structure :

Nystatin A_1 (or referred to as nystatin) is biosynthesized by a bacterial strain, Streptomyces noursei. The structure of this active compound is

characterized as a polyene macrolide with a deoxysugar D-mycosamine, an amino glycoside.The genomic sequence of nystatin reveals the presence of the polyketide loading module and two thioesterase. Thus, it is evident that the biosynthesis of the macrolide functionality follows the polyketide synthase I pathway

Mechanism of action:

Like amphotericin B and natamycin, nystatin binds to ergosterol, which is a major component of the fungal cell membrane. When present

- With sufficient concentrations, it forms pores in the membrane that lead to K^+ leakage, acidification, and death of the fungus.
- Ergosterol, is unique to fungi, so the drug does not have such catastrophic effects on animals or plants. However, many of the systemic toxic effects of Nystatin are attributable to its effect on human cells via binding to mammalian sterols, namely cholesterol.
- This is the effect that accounts for the nephrotoxicity observed when high serum levels of Nystatin are achieved.

Uses:

Candidiasis involving skin, vaginal mucosa and esophagus usually respond well to treatment with nystatin. It is available in many forms. Oral nystatin tablets is often used as a preventative treatment in risky individuals

for fungal infections, such as HIV positive individuals with a low $CD4^+$ count and patients receiving immunosuppressive drugs.

It is also used in Very Low Birth Weight (<1500 g) infants as prophylaxis to prevent invasive fungal infections, though fluconazole is the preferred agent. The drug also reduce mortality when used in such babies. Current clinical guidelines states that the use of these agents should be restricted to extremely low birthweight infants (<1000g) in NICU with fungal infection.

Investigational use of Liposomal Nystatin has shown greater *in vitro* activity than colloidal formulations of Amphotericin B, and found effective against some Amphotericin B resistant fungal infections. It is also used to treat systemic infections, such as invasive aspergillosis. Liposomal Nystatin appears to cause less chance of severe nephrotoxicity than observed with Amphotericin B.

Formulations :

- An oral suspension form is used for the prophylaxis or treatment of oropharyngeal thrush .A tablet form is preferred for candidal infections of GIT
- It is also available as a topical cream and can be used for superficial candidal skin infections.

- A liposomal formulation of nystatin was investigated in the 1980s and into the early 21st century and was intended to resolve problems arising from the poor solubility of the parent molecule and the associated systemic toxicity of the free drug.

Adverse effects:

The oral suspension produces a number of adverse effects including

- Abdominal pain
- diarrhoea
- Rarely, tachycardia, facial swelling, bronchospasm and myalgia Both the oral suspension and the topical form can cause:

1. Hypersensitivity like Stevens-Johnson syndrome in few cases
2. Rash, burning sensation are common with topical forms

Spectrum of Antifungal action:

Azoles as a group have clinically useful activity against Candida albicans, Candida tropicalis, Candida parapsilosis, Candida glabrata, Cryptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum, Coccidioides spp., Paracoccidioides brasiliensis, and ringworm fungi (dermatophytes). Aspergillus spp., Scedosporium apiospermum (Pseudallescheria boydii), Fusarium, and Sporothrix schenckii are

intermediate in susceptibility. Candida krusei and the agents of mucormycosis are more resistant. Posaconazole has slightly improved activity in vitro against the agents of mucormycosis.

Resistance:

Widespread use of azoles resistance has lead to the emergence of both primary and secondary resistance, causing clinical failure with advanced HIV infection and esophageal or oropharyngeal candidiasis. The main mechanism of resistance in Candida albicans is the accumulation of mutations in *ERG11*, the gene which codes for the 14-alpha-sterol demethylase. The above mutations protect heme in the enzyme pocket from binding to the azole but allow access of the natural substrate for the enzyme lanosterol.

Cross-resistance is seen in all azoles. The azole efflux by both ATP-binding cassette (ABC) and major facilitator transporters can enhance fluconazole resistance in Candida albicans and Candida glabrata. Excess production of C14-alpha-sterol demethylase is another potential factor of resistance. C5,6 sterol reductase gene *ERG3* mutation also can increase azole resistance in some species.

Antifungal susceptibility:

It is designed to provide information that helps the treating physician to select the appropriate antifungal agents. Isolates of same species may exhibit differences in MIC because of previous exposure to antifungals or genetic mutations. AmphotericinB, 5fluorocytosine, ketoconazole, itraconazole, voriconazole and fluconazole are common antifungals that are tested. Now newer antifungals are added to this list. National committee for clinical laboratory standards(NCCLS) has defined a standard for antifungal sensitivity testing. But its use in the clinical aspects are very limited because all azole resistant candidiasis cannot be explained by invitro methods. Resistance is more common with non Candida albicans species. Increasing the dosage of currently using drug, changing new drug, using combination of drugs are the alternate option for the above problem.

Two most common methods are used for susceptibility.

- Micro dilution method
- Disc diffusion method

Treatment regimens of VVC:

The cell wall of Candida is a complex glycoprotein that depends on the biosynthesis of ergosterol. Azole compounds, found in antimycotic drugs, are believed to block ergosterol production, allowing topical

antimycotics to achieve cure rates in excess of 80%. The only oral azole agent approved for this indication by the US Food and Drug Administration (FDA) is fluconazole, which also shows a high cure rate. Adequate therapeutic concentrations are found in vaginal secretions for 72 hours after the ingestion of a single 150-mg tablet.

In considering treatment, distinguishing between sporadic and recurrent episodes of vulvovaginal candidiasis is of great importance. Uncomplicated sporadic VVC is caused by C.albicans. These strains exhibit sensitivity to azole antifungals.

Studies have shown there is no marked difference in the clinical efficacy of various topical azole antifungals. So starting empirical treatment without waiting for culture results is advisable. In uncomplicated cases treatment is selected according to patient's preferences.

Ample number of antifungal regimens are available for the treatment of vulvovaginal candidiasis including oral and topical agents. But drug interaction with oral usage must be taken in to consideration. Hepatotoxicity secondary to ketoconazole therapy occurs in approximately 1 in every 10,000-15,000 individuals exposed to this drug. Side effects include nausea, abdominal pain, and headaches can also occur. Drug interactions occur with simultaneous use of warfarin ,calcium channel antagonists, theophylline, rifampicin, cyclosporine, oral hypoglycemic agents, protease inhibitors and to name a few.

In comparative trials of 10-14 of course of therapy azoles scored higher clinical cure rate (80 -95%) than another effective topical agent, nystatin (70-80%). Azoles found to be more efficacious even if given for less than 14 days.

CDC – guide lines 2010

1. Clotrimazole 1% cream 5g intra vaginally daily for 3 days

   Clotrimazole 100 mg vaginal tablet daily for 3 days
2. Butoconazole 2% cream 5g intravaginally daily for 3 days
3. Miconazole 2% cream 5g intravaginally for 7days(or)

   Miconazole 1200 mg vaginal supporitony -10 D× 1 day (Or)

   Miconazole 200mg vaginal supporitory Od for 3 days (or)

   Miconazole 100 mg vaginal supporitory 1 od for 7 days
4. Tioconazole 6.5% ointment 5g intravaginally in a simple application
5. Butoconazole 2 % cream- single dose, bioadhesive product 5g gingivally for 1 day.
6. Nystatin 1,00,000 unit vaginal tablet 10 D for 14 days (or)
7. 0.4 %Terconazole cream 5 g intravaginally for 7 days (or)

   0.8 %Terconazole cream 5 g intravaginally for 3 days (or)

   Terconazole 80mg vaginal supporitory for 3 days

Oral Agents

T. Fluconazole 150 mg single dose.

WHO guidelines(2003)

1. Miconazole (or) clotrimazole 200 mg pessary intravaginal daily for 3 days (or)
2. Clotrimazole 500 mg pessary intravaginally in a single dose or
3. T.Fluconazole 150 mg orally as a single dose

Alternative regimen

Nystatin 100000 unit vaginal tablet OD for 14 days

Other Regimens:

- Clotrimazole 500 mg intravaginally single application
- Clotrimazole 200 mg intravaginally for 3 days
- Tioconazole 2% cream 5 gm intravaginally for 7 days

For recurrent VVC

Several studies have shown that antifungal maintenance suppressive therapy for several months is effective in RVVC.

Some regimens includes:

1. Tab .ketoconazole 400 mg per week
2. Tab. Itraconazole 50-100 mg per week or 400mg monthly
3. Tab .Fluconazole 100 mg per week
4. Clotrimazole 500 mg vaginal suppositories once per week

The above regimens are used upto six months.

The cure rates for vulvovaginal candidiasis is72% to 98% in most of the studies done on patients without HIV infection. Previously, the standard regimen for vulvovaginal candidiasis typically consisted of topical forms of clotrimazole or miconazole for 7 days. However, shorter courses are proved to be equally effective. Response to topical treatment for 3 days is equivalent to topical therapy for 7 days. In a study comparing a single dose of fluconazole 150 mg orally vs 7 days of topical clotrimazole therapy with100-mg vaginal suppositories, the clinical cure rate (75%) in the 2 groups was equivalent by day 35. Mycologic eradication rates were 63% for the fluconazole group at day 35 and for the clotrimazole group it was 57%. Either topical or systemic therapy is effective in women with HIV infection, but relapse rates may be quite more.

Most of the Candida infections respond to empiric antifungal therapy. The reliability of invitro testing for antifungal resistance is less when comparing to antibiotic susceptibility testing of bacterial isolates.

Non albicans VVC

It includes longer duration of therapy (7–14 days) with preferably a nonfluconazole azole drug either oral or topical as first-line therapy. In case of recurrence , 600 mg of boric acid in gelatin capsule form is advised. It has to be administered intravaginally once daily for 2 weeks. This regimen has shown 70% clinical and mycologic eradication rates.

Topical boric acid has been used for long time as a treatment for vulvovaginal candidiasis. Though it is effective against non candida albicans infection, it is categorised as a poison and may be absorbed through the vaginal mucosa and reaches significant serum levels.[53] For protection, it is encapsulated in a gelatinous capsule and administered as a suppository. Treatment includes 600 mg in size 0 gelatin capsules administered intravaginally every day for 2 weeks.

Preventive measures of Complicated VVC:

- As ingested sucrose and lactose may support and promote the growth of yeast, the patients should limit their dietary intake of such sugars .
- Patients are also advised to wear loose-fitting, nonocclusive clothing and cotton underwear to avoid providing the warm, moist climate in which Candida tends to thrive.
- Some recommend using panty liners and washing clothing in hot water to avoid creating a reservoir for the fungus
- Adequate glycemic control should be maintained in diabetic patients.
- Cessation of OCP's results in reduction in the frequency of clinical episodes.
- Repeated unnecessary antibiotic intake should be avoided.

Sober's multi center prospective randomized study on maintenance of Fluconazole therapy in RVVC in 387 women , half were put on placebo and other half put on Fluconazole 150 mg induction, followed by 150 mg pulses weekly. After six months 98.5% were disease free; at nine months 73.2%, at 12months 42.9% were disease free.[54]

Observation and results

A total of 80 symptomatic female patients above 18 years who fulfilled the inclusion criteria were screened. Diagnosis of vulvovaginal candidiasis was confirmed in 50 case and they were taken for culture and sensitivity testing. The results we obtained is analysed and summarised in this section. All the results obtained were analysed statistically for their completedness, consistency and accuracy.

TABLE 1:

Age wise distribution of VVC

Age	Number of patients	Percentage
18 – 35 years	44	55%
36 -50 years	31	38.7%
>50 years	5	6.3%

The 80 study subjects were analysed based on age and tabulated(table1). Of the 80 patients , 44(55%) belonged to18-35 years; 31 patients (38.7%) belonged to the age group of 36 – 50 years and only 5 patients (6.3%) were in the age group of 50 and above. So majority of the patients fall in the reproductive age group (18-35years) were vulnerable to get VVC.

CHART :1

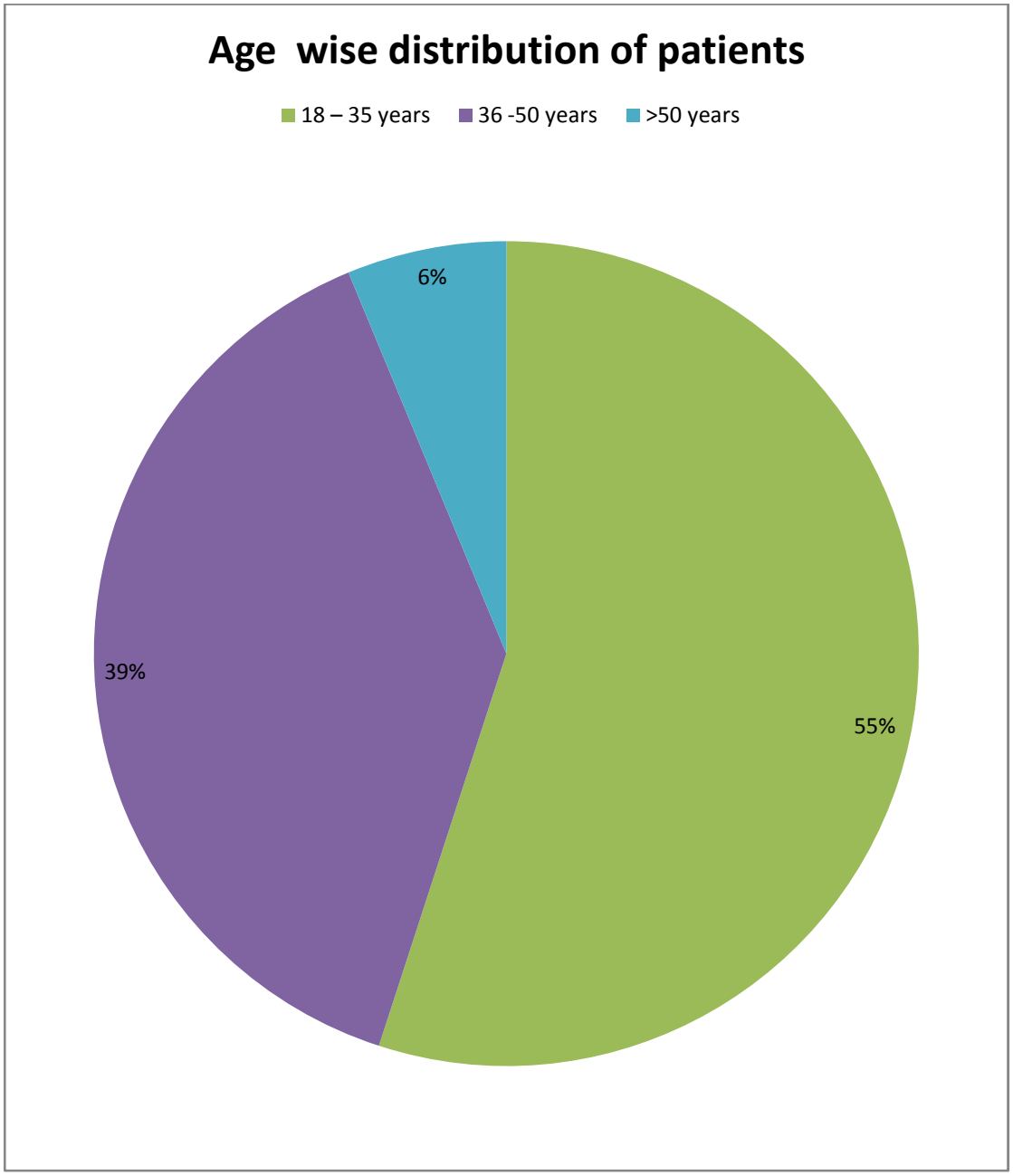

Age wise distribution of patients
18 – 35 years
36 -50 years
>50 years
6%
39%
55%

TABLE - 2

Analysis of clinical pattern of VVC

CLINICAL FEATURES	NO. OF PATIENTS	HIV STATUS		SPECIES	
		Positive	Negative	C. Albicans	Non C. Albicans
Asymptomatic cases	2	1	1		
Itching of vulva	78	38	40		
Scanty discharge	19	6	13		1
Profuse curdy white precipitate	51	29	25	43	8
Soddening of vulva	2	2			
Maceration of vulva,adjacent sites	8	2	6	6	2
Satellite pustules	1	1			
Erosion ,inflammed fissuring	6	1	5	3	3

All the study group patients were symptomatic except 2 patients. 19 patients had scanty vaginal discharge. Among this,13 were HIV negative. 51 patients had presented with profuse vaginal curdy white discharge amongst which 29 patients were HIV positives and 25 were HIV negative. 2 HIV positive patients showed soddening. Maceration of vulva and adjacent skin was seen in 8 patients and 6 of them were HIV negatives. Erosions, glazened appearance and fissuring was seen in 6 patients, five of them were HIV negative patients. Satellits pustules over the groins was seen in one patient only.

Among non Candida albicans species,all had itching and profuse vaginal discharge. Six patients had erosions, maceration and glazened appearance of the adjacent skin.

TABLE:3

Comparison of VVC and associated vaginal infections

Associated infections	Number of patients	Percentage
Bacterial vaginosis	5	6.25%
Trichomonas vaginalis	4	5%
Herpes genitalis	3	3.75%
Genital warts	1	1.25%
No association	22	27.5%

Among the 80 study subjects, 5 patients were coinfected with bacterial vaginosis(6.25%); 4 patients were coinfected with Trichomonas vaginalis(5%). Coexistent herpes genitalis was seen in 3 patients (3.75%). Genital warts were seen in only one patient(1.25%). The remaining 22 patients were not associated with any other infections.

CHART :2

Comparison of VVC and associated vaginal infections

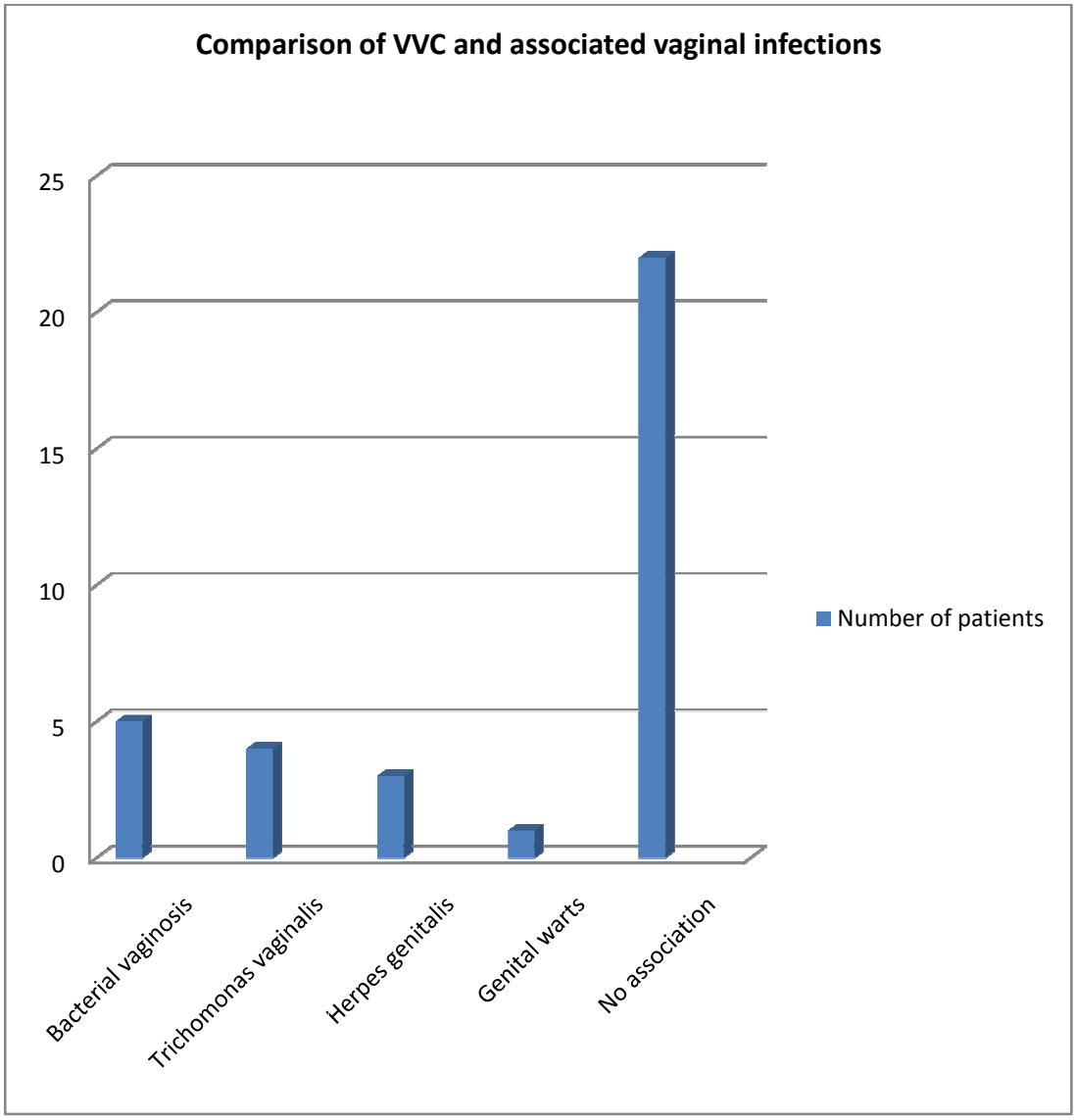


TABLE :4

Analysis of predisposing factors for VVC:

Precipitating factors	Number of patients	Percentage
Antibiotics usage	8	10%
Intrauterine devices	2	2.5%
Immunosuppressive drugs	2	2.5%
Diabetes mellitus	6	7.5%
HIV	39	48.7%

Of the 80 women,8 patients (10%) had H/O antibiotics usage in the recent past for some respiratory illness. Two patients (2.5%) presented with IUCD in situ, another 2 patients(2.5%) were on immunosuppressive drugs; 6 patients (7.5%) were diabetic not under glycemic control and 39 out of 80 were HIV positive(48.7%).

CHART :3

Analysis of predisposing factors for VVC:

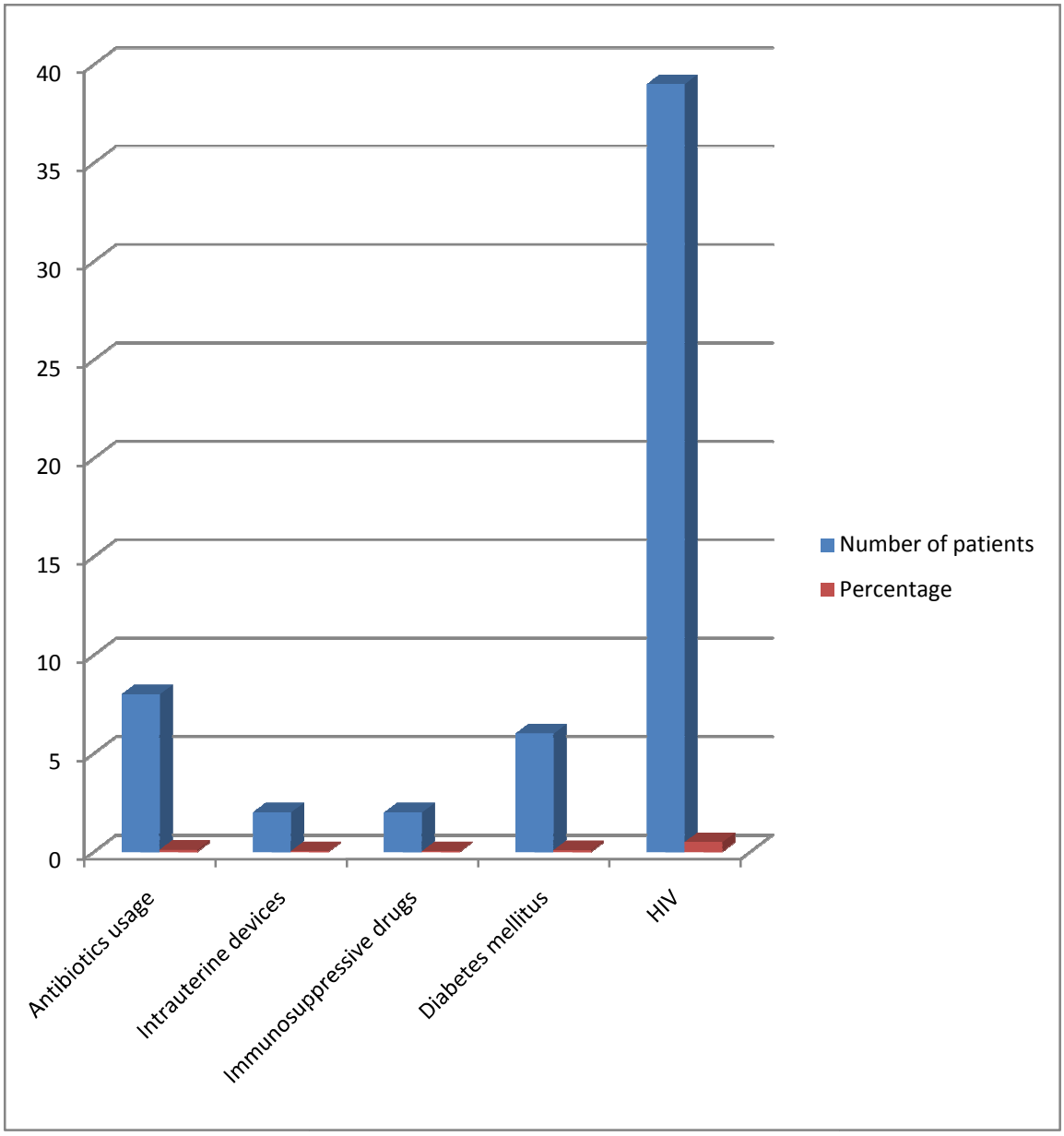


TABLE:5

Analysis of relationship between RVVC and HIV positivity

HIV status	Number of patients	Percentage
Positive	6	60%
Negative	4	40%

We have identified 10 cases of RVVC in the study group. Among the 10 cases, 6(60%) are HIV positive and 4 cases (40%) are HIV negative. Among the HIV negatives, 2 cases are diabetics and 2 cases are normal subjects. Their CD4 counts were in the range of 150 to 400 cells/µL.

CHART :4

Analysis of relationship between RVVC and HIV positivity

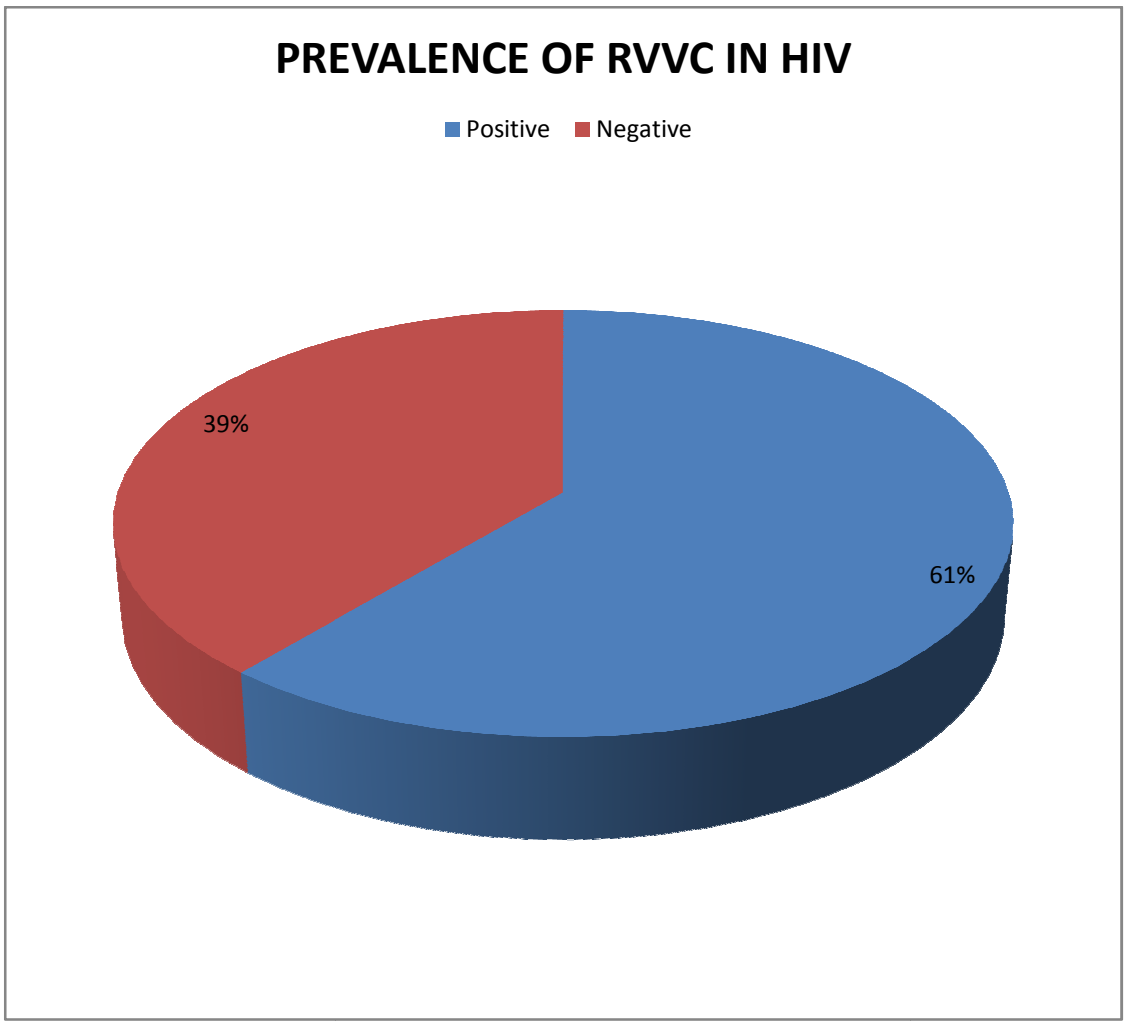


TABLE: 6

Identifying species prevalence in the study group

Species	Number of patients	Percentage
Candida albicans	41	82%
Candida glabrata	4	8%
Candida krusei	2	4%
Candida tropicalis	3	6%

Among 80 patients, 50 are confirmed cases of VVC by culture in SDA agar and Chrome agar. Among the culture positive cases ,41cases (82%) are Candida albicans; 4 cases (8%) are C.glabrata; 2 cases (4%) are C.krusei and 3 cases (6%) are C.tropicalis.

CHART :5

Identifying species prevalence in the study group

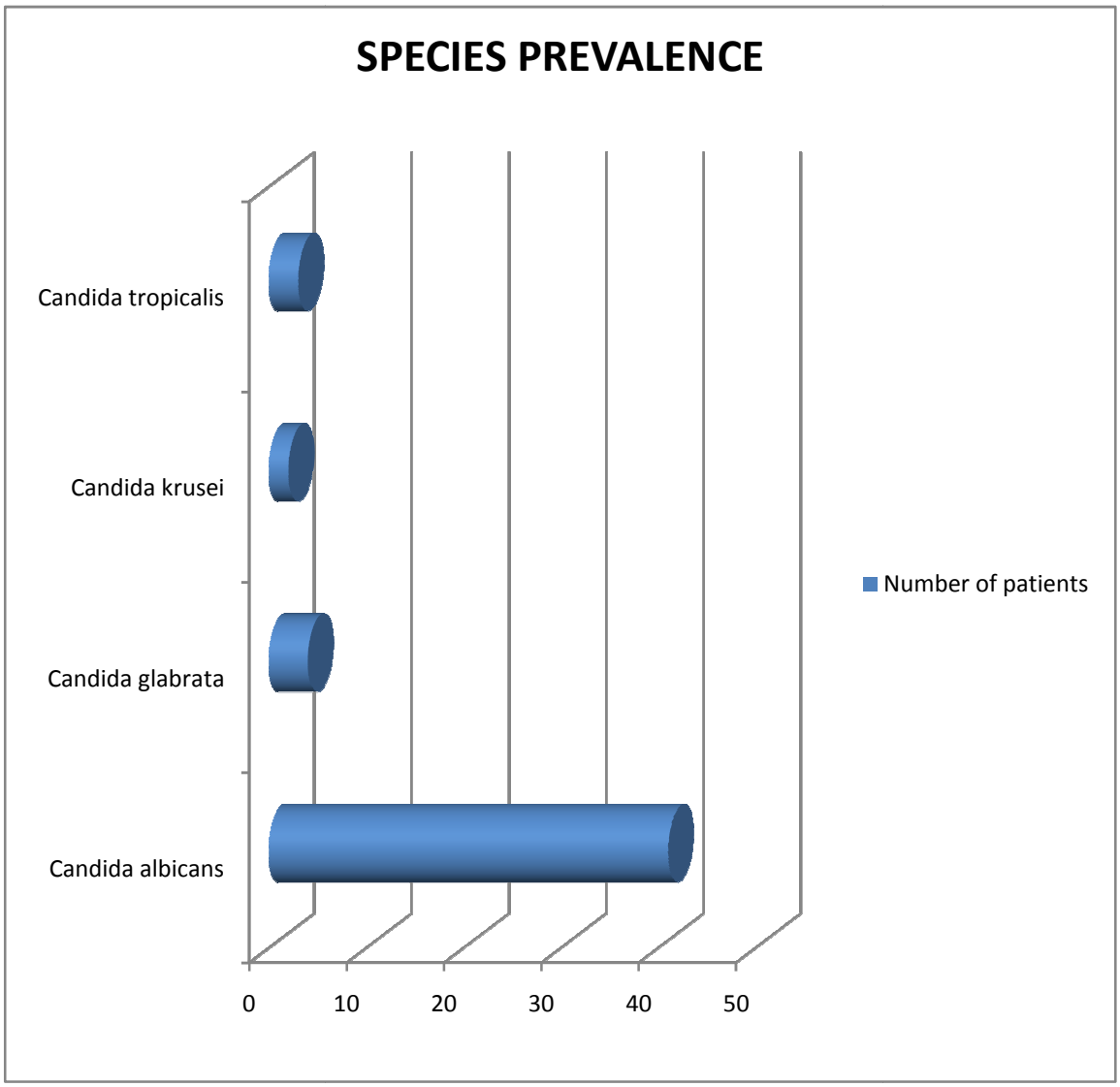


TABLE :7

Analysis of drug sensitivity pattern in VVC

Drugs	Sensitive	Percentage	Resistant	Percentage
Fluconazole	41	82%	8	16%
Itraconazole	40	80%	8	16%
Nystatin	45	90%	3	6%
Clotrimazole	37	74%	6	12%
Voriconazole	28	56%	15	30%
Miconazole	26	52%	13	26%
Ketoconazole	20	40%	23	46%

Of the 50 culture positive cases, the isolates were subjected to drug sensitivity by disc diffusion method.45 cases (90%) were sensitive to nystatin; 41 cases(82%) are sensitive to fluconazole;40 cases(80%) were sensitive to itrconazole;37 cases(74%) were sensitive to clotrimazole other drugs like ketoconazole, miconazole, voriconazole were less sensitive.

CHART: 6

Analysis of drug sensitivity pattern in VVC

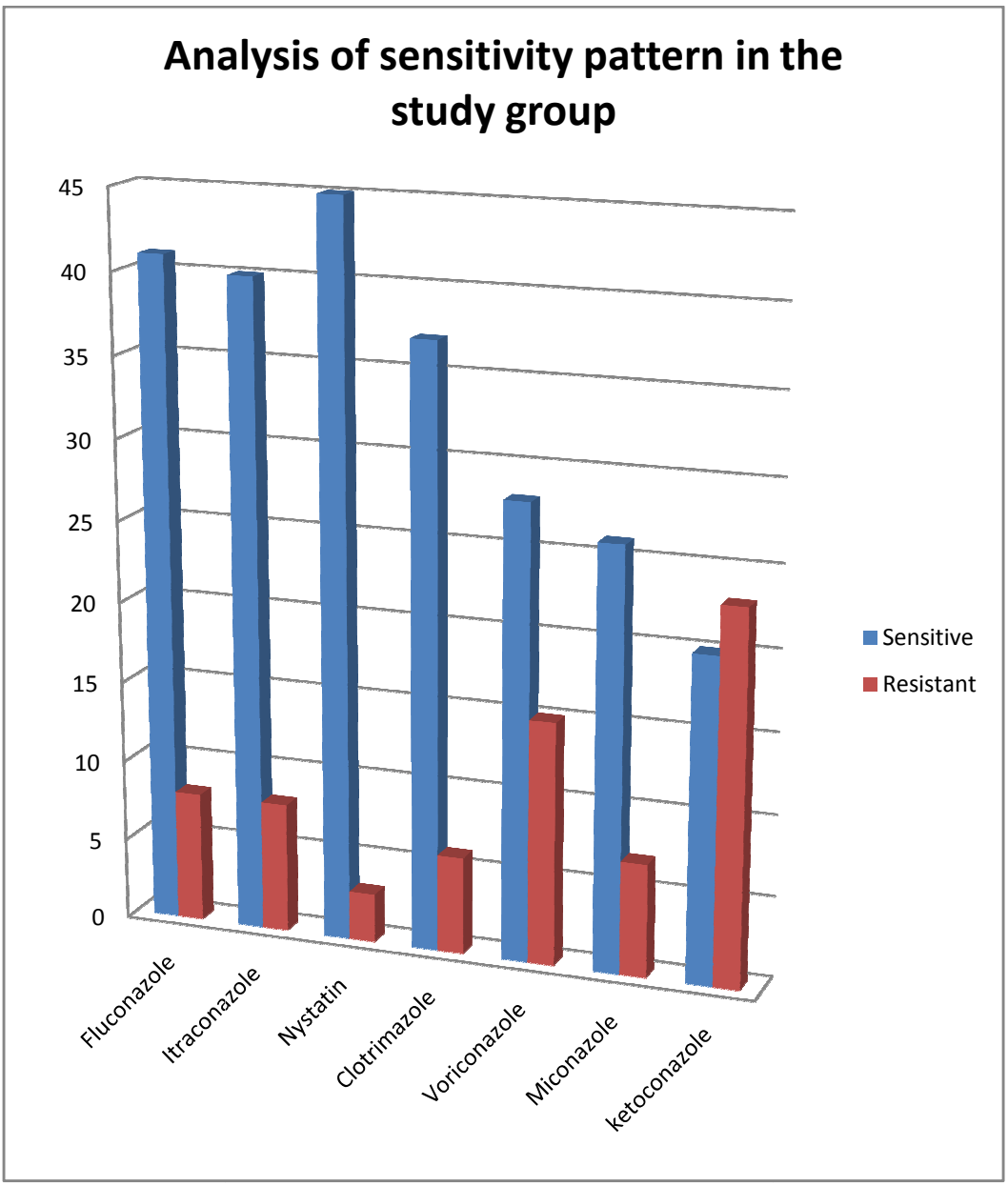


TABLE: 8

Analysis of drug sensitivity pattern in Candida albicans

Drugs	Sensitive	Percentage	Resistance	Percentage
Fluconazole	34	82%	7	18%
Itraconazole	35	85.3%	6	14.7%
Nystatin	36	87.8%	5	12.2%
Clotrimazole	31	75%	10	25%
Voriconazole	31	75%	10	25%
Ketoconazole	16	39%	25	61%
Miconazole	22	53%	19	47%

Among the 41 proved cases of Candida albicans, Fluconazole, Itraconazole, Nystatin were highly sensitive drugs (more than 80 %). 36 cases were sensitive to nystatin; Itraconazole was sensitive in 35 patients; Fluconazole was sensitive in 34 patients(82%) and voriconazole was sensitive in 31(75%) patients. Others like clotrimazole, ketoconazole, miconazole were sensitive in 31(75%),16(39%), 22(53%) patients respectively.

CHART - 7

Analysis of drug sensitivity pattern in Candida albicans

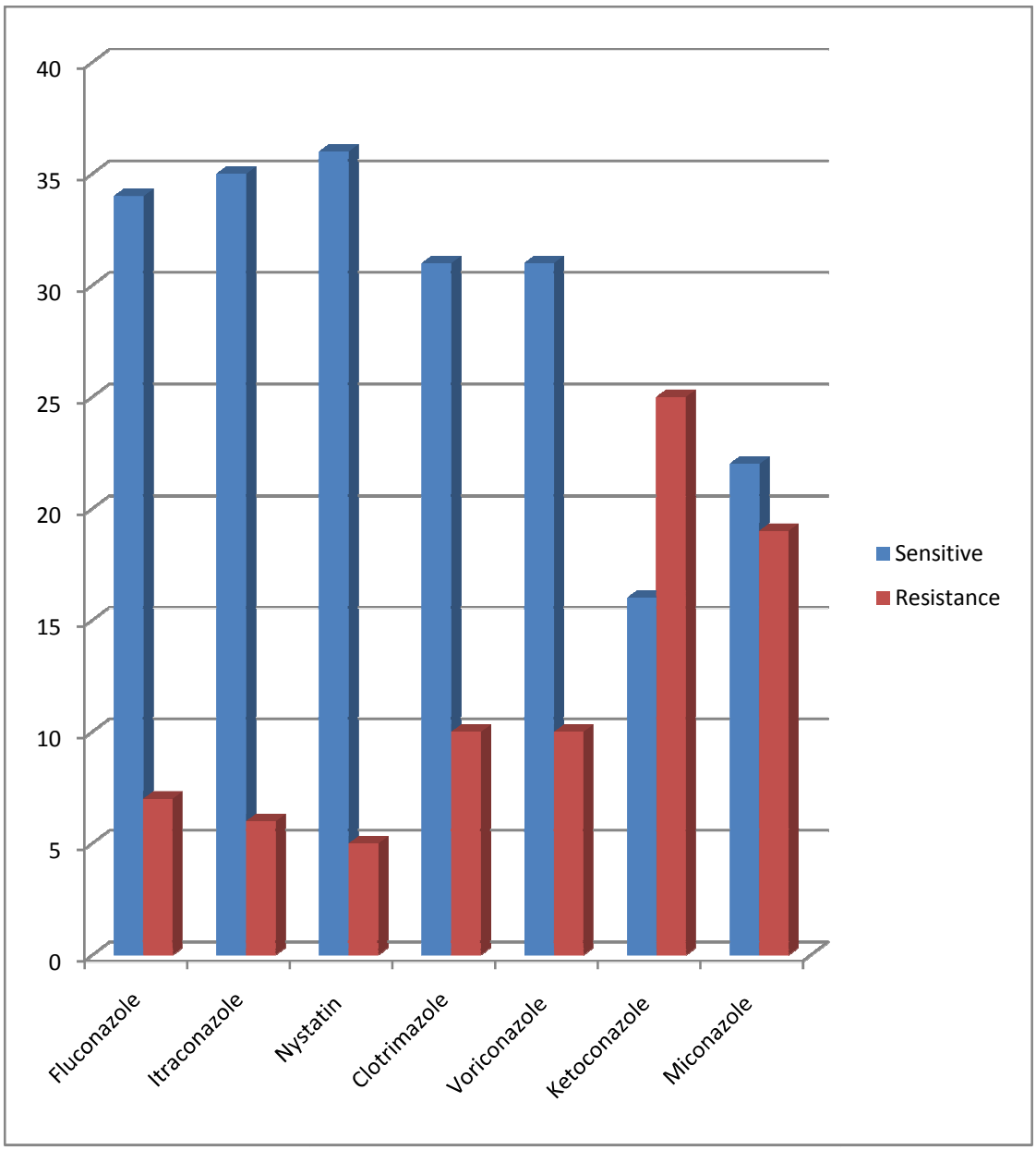


TABLE - 9

Drug sensitivity pattern in non Candida albicans patients

Drugs	Sensitivity (No of patients)	Percentage	Resistance (No of patients)	Percentage
Fluconazole	7	77.7%	2	22.3%
Itraconazole	6	66.6%	3	33.3%
Nystatin	9	100%	0	0
Clotrimazole	7	77.7%	2	22.3%
Voriconazole	7	77.7%	2	22.3%
Ketoconazole	5	55.5%	4	44.5%
Miconazole	7	77.7%	2	22.3%

Among the 9 nonCandida albicans group all were sensitive to nystatin(100%). Fluconazole , clotrimazole, voriconazole and miconazole showed sensistivity in 7 (77.7%) patients. Six patients (66.7%) were sensitive to itraconazole. Ketoconazole was least sensitive (55%).

CHART - 8

Analysis of sensitivity pattern in non candida albicans group:

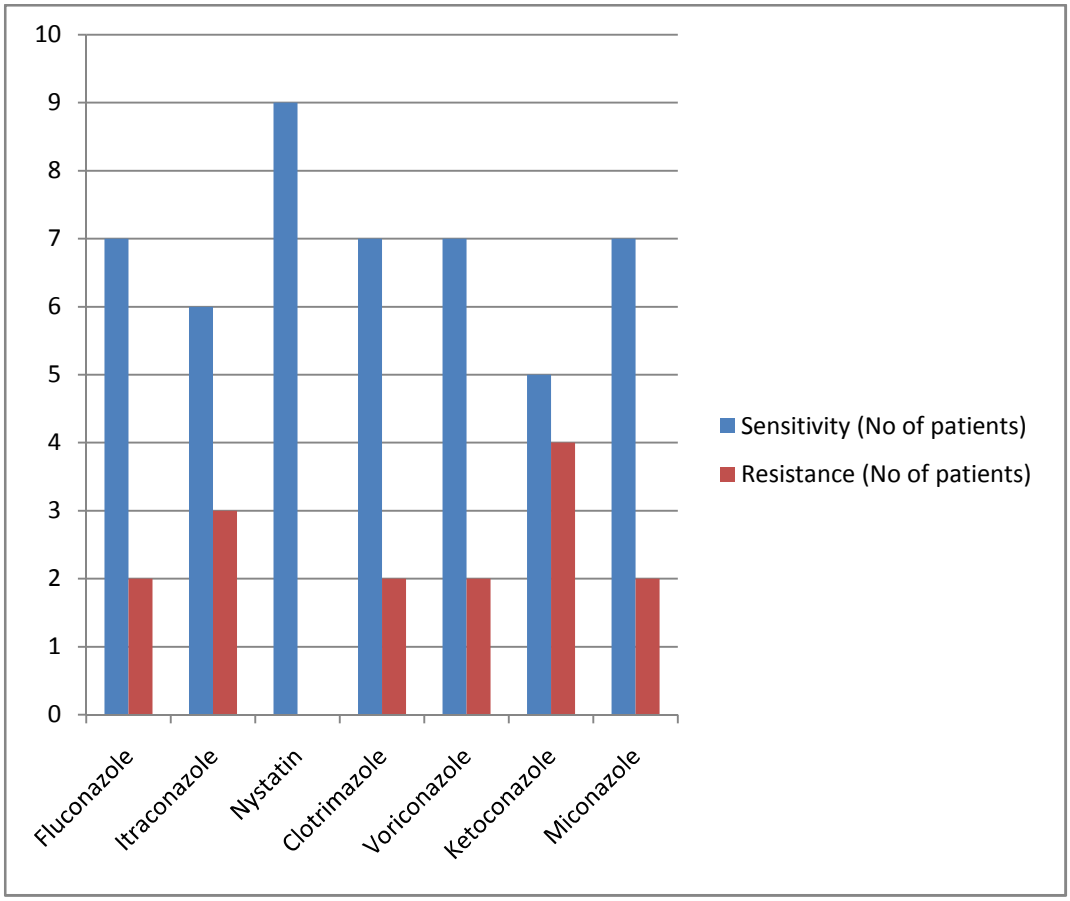


TABLE 9(A):

C. glabrata

Drugs	Sensitive	Resistance
Fluconazole	3	1
Itraconazole	2	2
Nystatin	4	0
Clotrimazole	2	2
Voriconazole	3	1
Ketoconazole	3	1
Miconazole	3	1

Among 4 cases of candida glabrata , 3cases showed sensitivity to fluconazole ,voriconazole ,miconazole and one case was resistant to fluconazole. Itraconazole was sensitive in 2 cases and resistant in 2 cases.

TABLE 9(B)

Drug sensitivity in Candida tropicalis

Drugs	Sensitive	Resistance
Fluconazole	3	0
Itraconazole	3	0
Nystatin	3	0
Clotrimazole	3	0
Voriconazole	3	0
Ketoconazole	2	1
Miconazole	2	1

All the 3 cases of candida tropicalis were sensitive to fluconazole, itraconazole and nystatin. One case was resistant to clotrimazole and miconazole.

TABLE :9 (C)

Drug sensitivity in Candida krusei:

Drugs	Sensitive	Resistance
Fluconazole	1	1
Itraconazole	1	1
Nystatin	2	0
Clotrimazole	2	0
Voriconazole	1	1
Ketoconazole	0	2
Miconazole	2	0

One out of two cases was sensitive to Fluconazole ,Itraconazole , Voriconazole and one case was resistant to the above drugs. Both 2 cases showed sensitivity to nystatin ,clotrimazole, ketoconazole and both 2 were resistant to miconazole.

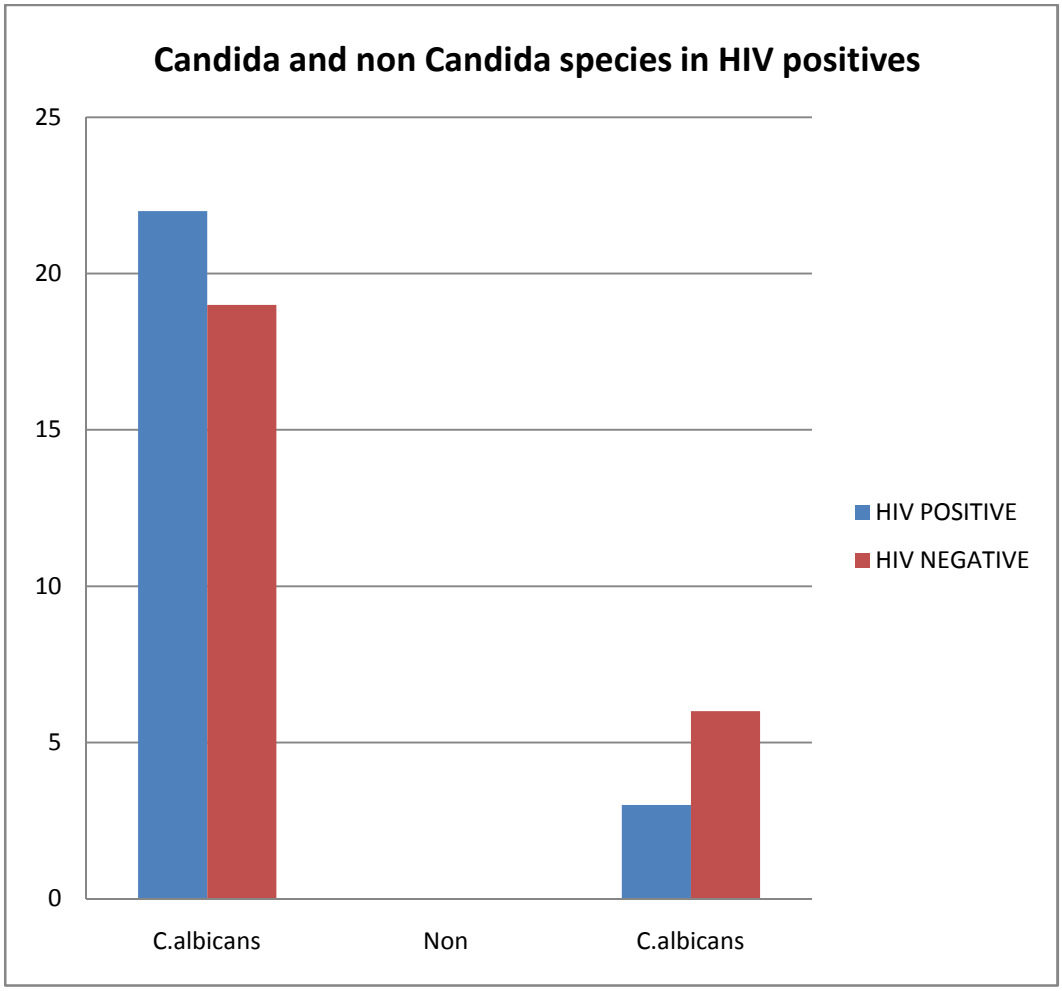
TABLE 10:

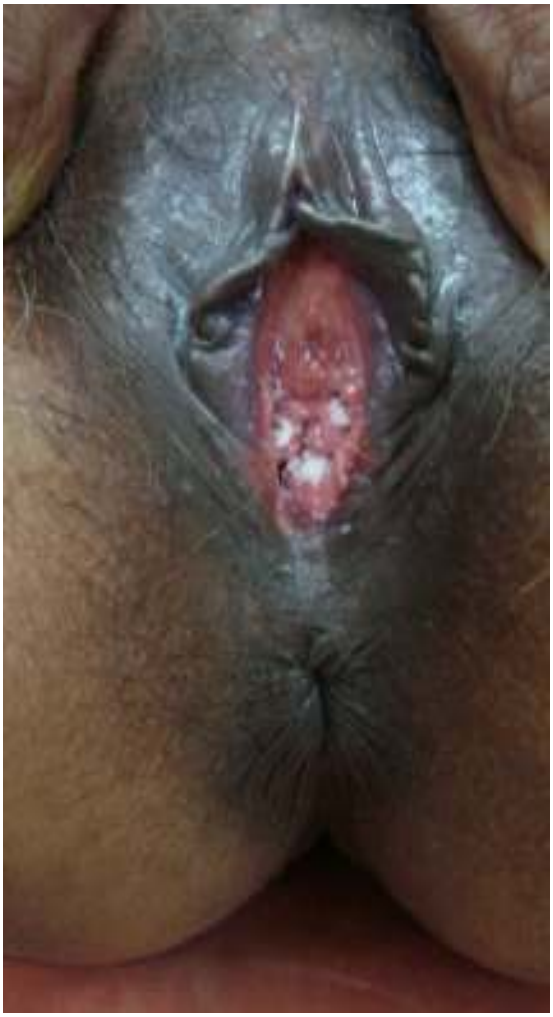
Prevalence of candida and non candida species in HIV positive patients

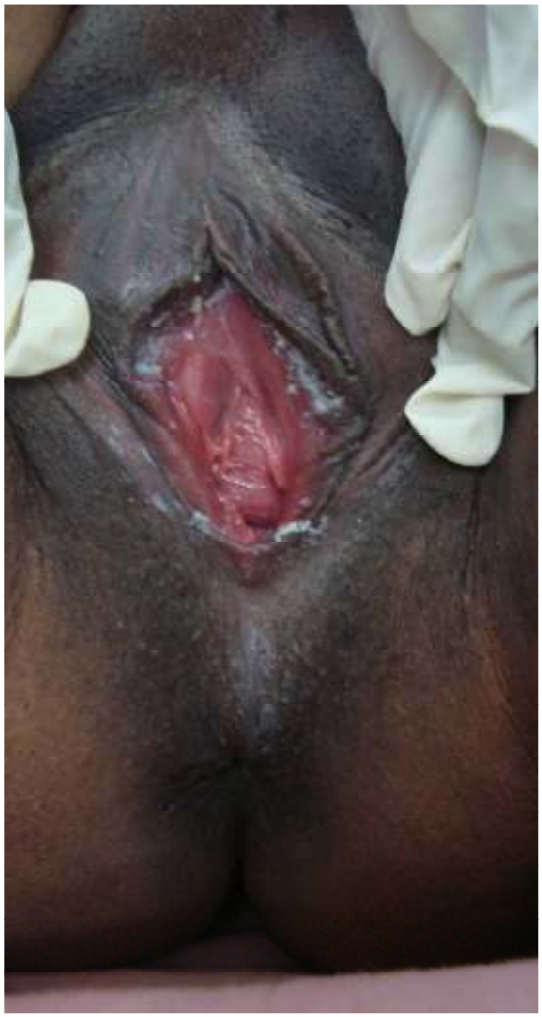
SPECIES	HIV POSITIVE	PERCENTAGE	HIV NEGATIVE	PERCENTAGE
C.albicans	22	53.6%	19	46.4%
Non C.albicans	3	33.3%	6	66.7%

Of the total 41cases of Candida albicans , 22 {56.4%} were HIV positive and 19(48.7%) were HIV negative. Among the 9 non candida albicans group, 3 cases (33.3%) were HIV positive and 6(66.7%) were HIV negative.

CHART -9


Candida and non Candida species in HIV positives
25
20
15
10
5
0
C.albicans
Non
C.albicans
HIV POSITIVE
HIV NEGATIVE





<u>Figure:1</u> - Curdy White Patches of Discharge in the inner aspect of labia minora & Fourchette

<u>Figure : 2</u> - Thick Curdy White discharge with inflammed vaginal mucosa in speculam examination

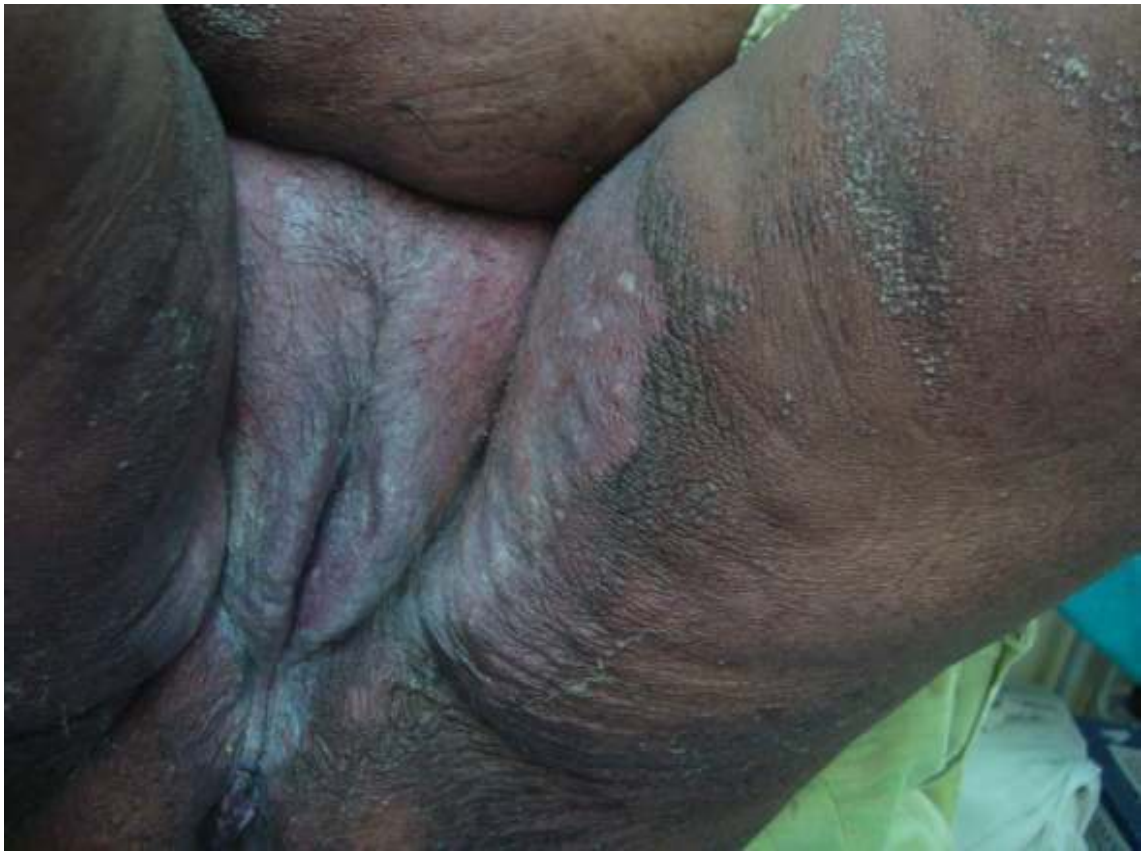


Figure : 3 – Maceration and Erosion involving Vulva and adjacent skin.

Figure : 4 – Soddening of Vulva and Intertrigenous Area

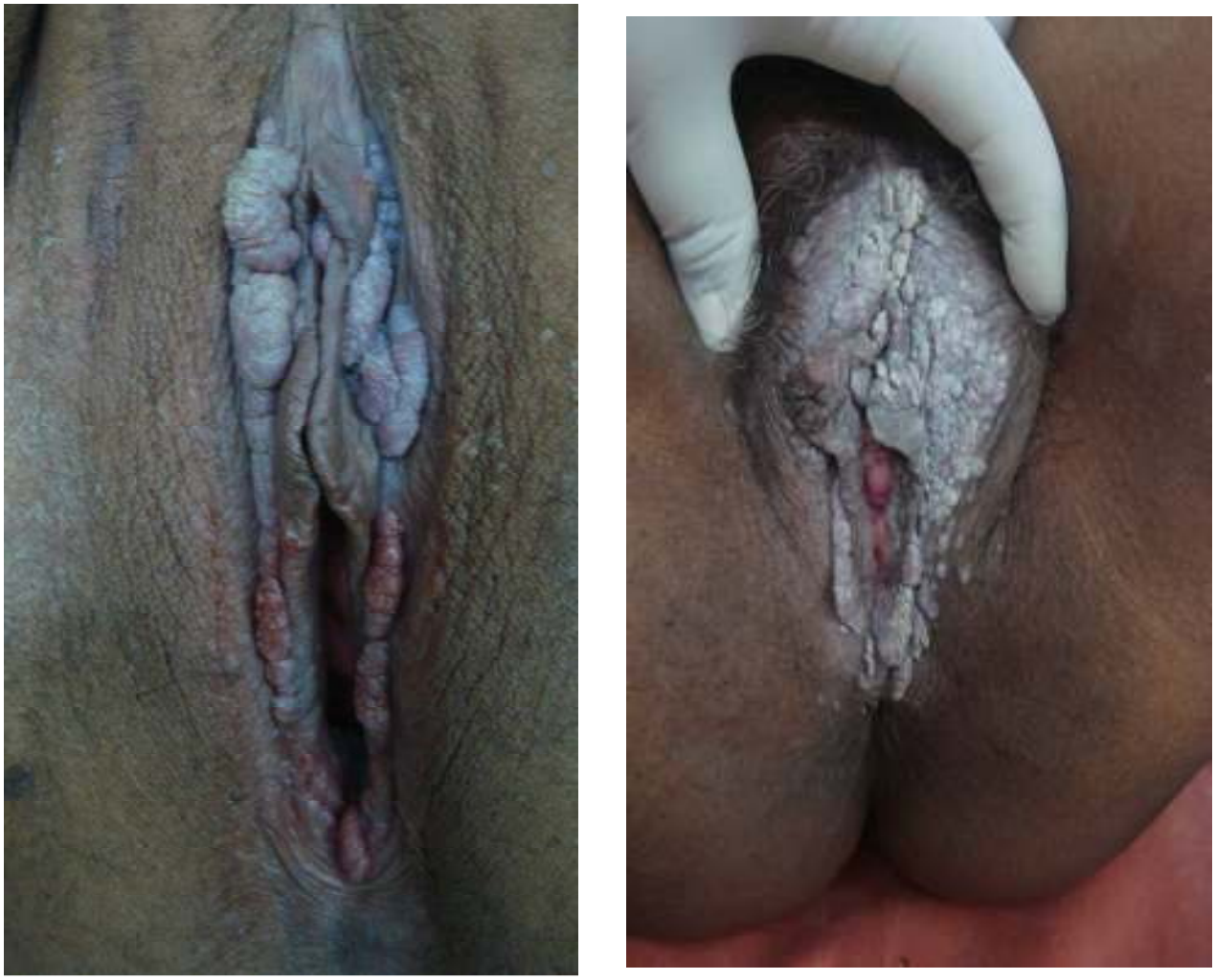


Figure : 5 – Vulvo Vaginal Candidiasis with Mutiple Genital warts

Figure : 4 – Large Herpetic Ulcer with secondary Candidiasis in the Anogenital Region.

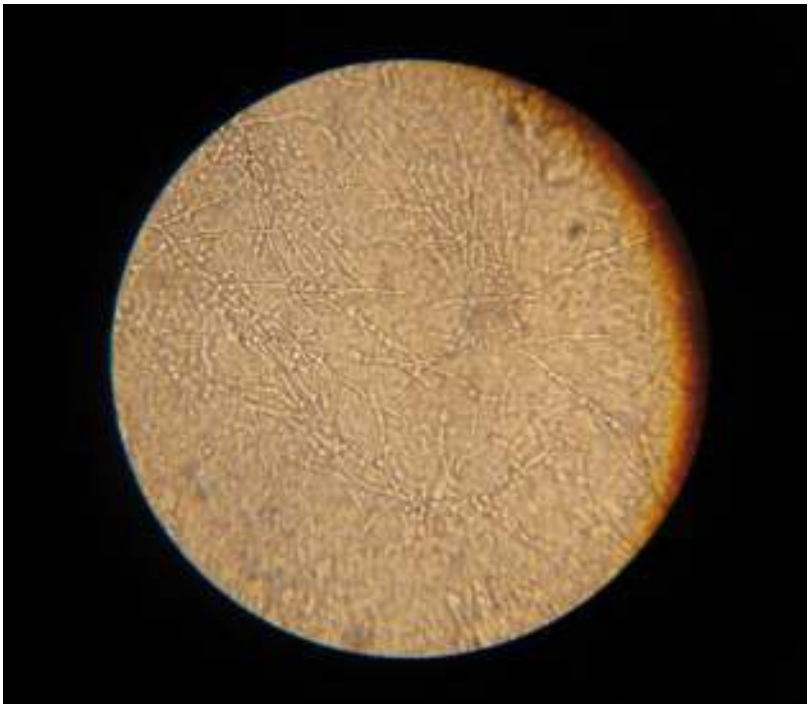


Figure :7 – KOH Mount of Vaginal Smear with Branched Hyphae & yeasts

Figure :8 – Sabourauds Dextrose Agar with smooth Creamy dome shaped colonies of Candida Albicans

Figure : 9 – HI chrome Agar Media showing smooth Green Coloured wet colonies of Candida Albicans

Figure : 10- HI chrome Agar Media showing pink coloured wet colonies of candida glabrata

Figure : 11- HI chrome Agar Media showing Metallic Blue Coloured Colonies of Candida Tropicalis

.

Figure : 12 - HI chrome Agar Media showing white to dull pink colour colonies of candida krusei

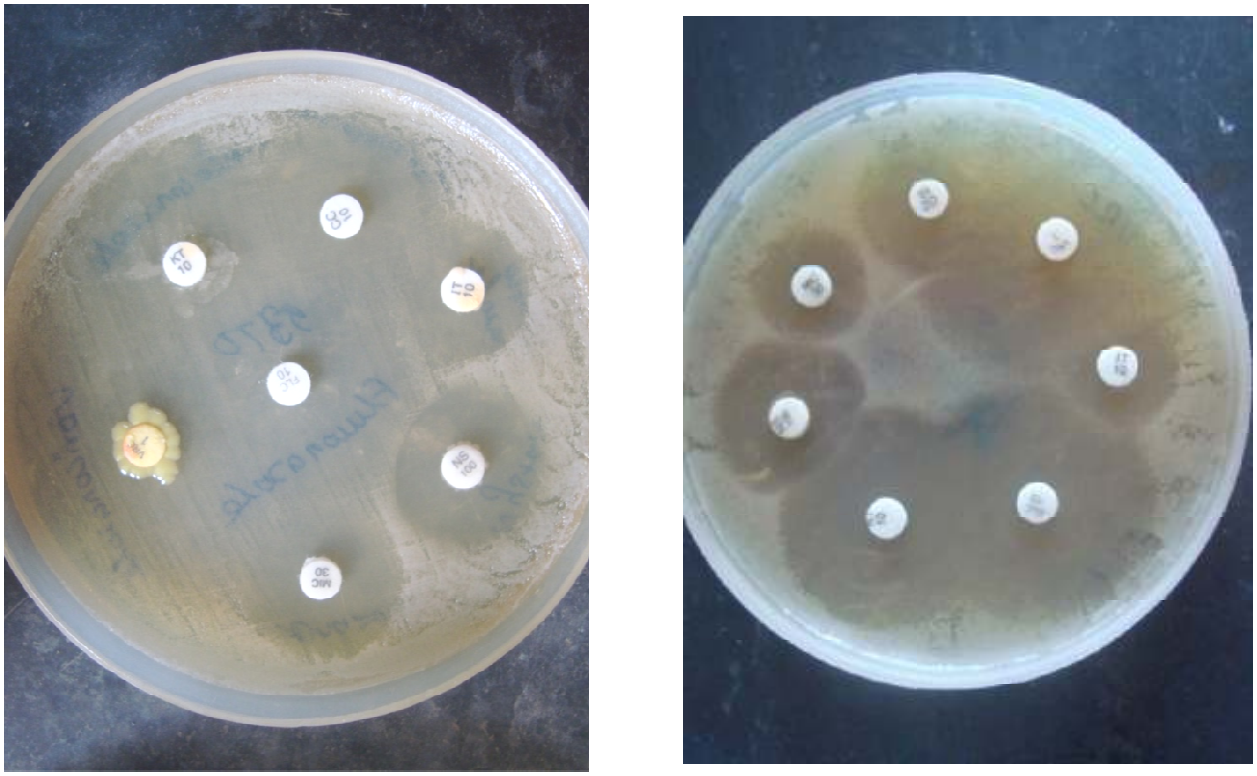


Figure : 13- Drugs Sensitivity by disc diffusion method showing sensitivity to All Drugs.

Figure : 14 - Drugs Sensitivity by disc diffusion method showing Multi Drug resistant Pattern

DISCUSSION

Vaginal candidiasis is a common infection in 60 to 70% of females of child bearing age. This study was done among the people attending the STD clinic of Dermato-Venereo-Leprology department with complaints of vaginal pruritus and discharge revealed highest frequency of vaginal candidiasis in the age group of 18 to 35 years which constitutes 55% followed by 36-50 years of age group(38.7%) as depicted in chart 1.Least frequency of around 6.3% is observed in menopausal age group. The studies done by A.K. Ako-nai, O.O.Kasimet al,Deepa babin et al[55] and Shegal et al[56] also showed the higher frequency of vulvovaginal candidiasis in the reproductive age group which was similar to our observation.

We have analysed the clinical presentation of VVC and correlated with HIV status and recurrence. Among the study group 97% were symptomatic patients. All had itching of the vulva and vagina. HIV negative and positive patients had more or less similar symptoms except for scanty vaginal discharge in 13 HIV negatives versus 6 HIV positives. One HIV positive case with recurrent VVC had severe symptoms with maceration and satellite pustules in the adjacent areas. But severe forms of VVC was more common in HIV negative patients infected with non Candida albicans species. Among non Candida albicans group, 2/3rd of the patients had profuse curdy white discharge with vaginal wall inflammation, erosions and

maceration of the adjacent areas. There is no much difference in the clinical features between HIV positive and HIV negative women as observed in study done by Singh S, Sobel JD, Wiesenfield HC, Marten's et al.[57]

We have analysed the predisposing factors for vulvovaginal candidiasis and tabulated (table 4). In our study HIV is the major predisposing factor. Out of 80 cases 39 were HIV positives. A study done by E.Shifrin et al[58], Duerr et al, A.Spinillo et al[63,64] published that VVC was one of the most common opportunistic fungal infection in HIV positive women because of depressed cell mediated immunity. Studies done by Ohmit SE, Schuman P et al also documented the same.

Frequent and prolonged antibiotic usage in the recent past was a major factor and it constitutes 10% in the total study group. Studies done by Spinillo A et al, Capuzzo et al,[59] Pirotta et al[60] documented that high prevalence of symptomatic vulvovaginal candidiasis was seen in frequent and prolonged antibiotic use. We observed the same finding in our study.

Co existing diabetes mellitus especially when uncontrolled has been the second most common risk factor in this study(7.5%). Similar observations were noted in studies done by De Leon et al[61] Dehemet elm et al, Jacober et al, Donderrs G[62] et al showing diabetes was the frequent predisposing factor for vulvovaginal candidiasis because hyperglycemia provides enrichment for the growth of the Candida in the vagina and also

resistant to antimycotics. In our study also 3 out of 6 diabetic patients(50%) are resistant to fluconazole and itraconazole.

We also correlated the IUCD and immunosuppressive drug intake in the causation of vulvovaginal candidiasis. Both factors carries the same weightage (2%) in the causation of vulvovaginal candidiasis. Barbone F'et al[65.] described the IUCD usage was associated with increased risk of infection. Apart from IUCD, oral contraceptives, diaphragm and spermicide also predisposes to vulvovaginal candidiasis (Hooten et al)[66].

We have analysed the coinfections with vulvovaginal candidiasis in our study group . The genital coinfections which occurred in higher frequencies were bacterial vaginosis (6.25%), trichomonas vaginalis (5%), herpesgenitalis (3.75%) and genitalwarts (1.25%) in decreasing order of frequency. 22 women (27.5%) had no associated infections. HIV is the most common association in vulvovaginal candidiasis.[67] 39 cases out of 80 cases are HIV positive and 9 out of 39 positive cases had oral candidiasis . Oropharyngeal and oesophageal candidiasis showed increased frequency in HIV positive patients was observed in studies done by Fitchenbaem et al.[67]

Regarding recurrent vulvovaginal candidiasis, we identified 10 cases out of which 6 cases were HIV positves and 4cases were HIV negative. Studies on recurrent vulvovaginal candidiasis done by Roads et al.[68] documented RVVC was common in HIV positive women and correlated the

impact on clinical and microbiological characteristics. He also stated that the incidence of RVVC correlate with advancing immunosuppression.

We analysed the most prevalent species among the culture positive cases and observed that 82% were C.albicans and 18% were non candida albicans(Spinilo et al). We isolated 9 cases of non candida species, of which 4 (8%) belongs to C.glabrata,3(6%) belongs to C.tropicalis, and 2 cases(4%) belongs to C.krusei. Reports from Uma et al,Deepa babin et al,Mahumodi et al,Fan et al,Paul nyiojery et al[69] have also documented similar results. In our study C.glabrata was most common followed by C.tropicalis and C krusei. The emphasize on non C.albicans was very essential because of drug resistance. Various study reports done by sobel et al,[69] Spiniliio et al, Redondo et al[70] were similar to our results. Hence speciation by reliable culture methods is beneficial in the diagnostic aspect.

In our study we correlated the prevalence of vulvovaginal candidiasis with HIV status of the patients. Of the 50 culture positive cases 22 out of 41cases of candida albicans were HIV positive(56.4%). Non candida albicans in HIV positives are 33.3%which was less than the cases found in HIV negatives(66.7%).Studies done by sober et al, Horowitz et al ,A Spinillo et al documented that non candida species were more prevalent in HIV positive patients. But our study results found that non candida albicans were more prevalent in HIV negatives only.

In vitro drug sensitivity testing has been improved over the past few years because increased incidence of primary and acquired resistance to azoles. Some studies documented that the invitro resistance to antifungal medication was common[71]. Although clinical and invitro drug resistance to candida albicans was uncommon, non candida albicans were less likely to respond to azole antifungal therapy[72].The rate of fluconazole resistance vary from 5-25%. But the rate of resistance was high in ketoconazole and itraconazole (5-56%). Similar observations were noted in our study results revealed highest susceptibility to nystatin (90%) followed by fluconazole (82%), itraconazole (80%), voriconazole (75%) clotrimazole(75%). Ketoconazole and miconazole were less sensitive. Regarding resistance pattern, ketoconazole (61%) was more resistant followed by miconazole(47%).

We also compared the susceptibility of C. albicans and non candida albicans drug sensitivity. For candida albicans, sensitivity to nystatin(87.8%) is high followed by itraconazole (85.3%), fluconazole(82%) clotrimazole(75%) and voriconazole(75%). Ketoconazole showed high level resistance.

Regarding noncandida albicans,3 out of 4(75%) candida glabrata showed sensitivity to fluconazole ,voriconazole ,miconazole and only 25%

was resistant to fluconazole. Itraconazole was sensitive in 2 cases(50%) and resistant in 2 cases(50%).

Drug sensitivity in Candida tropicalis showed all the 3 cases (100%) to be sensitive to fluconazole, itraconazole and nystatin and one case was resistant to clotrimazole and miconazole. Drug sensitivity in candida krusei, 2 cases showed 100% sensitive to nystatin. Azoles(fluconazole, itraconazole, voriconazole) were only 50% sensitive. Both the cases were resistant to miconazole. While comparing our results with other studies done on drug susceptibility pattern, all the three non C.albicans species showed highest sensitivity to nystatin.

Out of 9 cases of non Candida albicans all were sensitive to nystatin. 66 to 78% sensitivity was observed to fluconazole, voriconazole, clotrimazole and itraconazole. Ketoconazole showed least sensitivity (55%). Further analysis of drug sensitivity pattern in large study group is necessary. Our study showed similarity with a study done by Panchal et al[73,74] in western India.

We compared the drug susceptibility pattern of Candida albicans and non Candida albicans in this study which showed similar pattern of sensitivity and resitance in both groups. Nystatin scored the highest sensitivity followed by fluconazole, itraconazole and voriconazole. Ketoconazole showed highest resistance in both groups.

Summary

The following are the implications derived from this prospective study on clinicomycological study of vulvovaginal candidiasis among the patients more than 18 years of age over a period of one and a half years.

- The most common age group affected was 18 to 35 years which constitutes 55% of the study group
- Clinical presentation of VVC was similar in HIV positive and HIV negative patients. Severe forms were found in non Candida albicans and RVVC patients.
- The most common predisposing factor was HIV infection induced immunosuppression[48.7%] followed by antibiotic usage and diabetes mellitus.
- The most common associated infection was HIV [48.7%] followed by bacterial vaginosis and trichomonas vaginalis.
- Recurrent vulvovaginal candidiasis [RVVC] makes up 12% of total cases. Two third of them were HIV positives.
- The most common species isolated was Candida albicans which constituted 82% and the rest were non Candida albicans species.
- Candida glabrata was the most prevalent species among the non Candida albicans.

- Drug sensitivity pattern of Candida albicans showed highest sensitivity to Nystatin 85.7% followed by triazoles [75-85%]. Ketoconazole was more resistant[40-60%]
- Drug sensitivity pattern of non candida albicans showed 100% sensitivity to nystatin followed by triazoles [66-77%]. Ketoconazole showed highest resistance [45%]

Conclusion

Vulvovaginal candidiasis is common among women in the reproductive age group. Among various predisposing factors the deficient immune status of HIV predispose to pathogenic colonisation of vagina and frequent recurrences. Besides HIV some other factors like antibiotics, diabetes mellitus, IUCD usage and immunosuppressive drug intake also predisposes to vulvovaginal candidiasis. Species identification by culture methods showed Candida albicans to be the most prevalent species including the HIV positive patients in this study. Emergence of few nonCandida albicans species, recurrent vulvovaginal candidiasis necessitates the species identification and antifungal susceptibility to be done as a part of laboratory evaluation of vaginal candidiasis. Fluconazole , itraconazole and voriconazole were sensitive in both Candida albicans and non Candida albicans infections. Nystatin was found to be the highly sensitive drug followed by triazoles. Ketoconazole and miconazole were highly resistant to both Candida and non candida albicans species. According to our study results , elimination of predisposing factors and ensuring good immunity with identification of causative fungal species and treatment according to the drug sensitivity pattern will reduce the incidence of vulvovaginal Candidiasis.

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CLINICOMYCOLOGICAL STUDY OF VULVOVAGINAL CANDIDIASIS

PROFORMA

Sl.no: Date:

Patient Name:

Age : yrs

Sex : Male/Female

OP/IP No :

Address :

Phone No :

Complaints :

Itching over genital - +/- duration

Discharge pervaginum - +/- duration

History of present illness:

Itching over the genitals - +/- duration

Discharge pervaganum - +/- duration Nature

Foul smelling - +/-

Yeasty odour - +/-

Itching over the perineum- +/-

H/o recurrent attacks - +/- No.of episodes

Relationship to menstrual cycles -premenstrual /Menstrual/Postmenstrual

H/o dyspareunia /dysuria - +/-

H/o similar lesions in any other parts of the body - Oral /intertriginous

H/o spouse having similar complaints

Past history

H/o DM/HT/BA/TB/HIV-

H/o previous episodes

H/o obstetric/gynaec problem

Drug History:

Topical Application +/- details

Oral Medication +/- details

Other Medication +/- OHA
Immuno suppressants
OCP

Contact Histroy

General Examination

Conscious ☐ Oriented ☐ Anaemia ☐ Jaundice ☐ Lymph denopathy ☐

Pulse Rate- /mt Blood pressure - /mmHg

CVS - RS P/A CNS

Local examination

Sites involved by curdy patches: Vulva/vagina/groins/Perianal

Macroscopic examination

Nature of discharge

Nature of Vulval Mucosa - Duskyred/white Soddened /excoriation/ ulcer

Nature of Surrounding skin

Speculum Examination:

Nature of vaginal mucosa -

Erosions +/- Ulcers - +/-

Oral Mucosa –

Other cutaneous lesions – Nails /inter trigenous areas

Investigations

- Blood - Urea
  sugar
  creatinine
- Complete hemogram
- Urine - Albumin
  Sugar
  Deposits
- Vaginal smear - KOH mount
  Wet mount
  Gram staining
- Culture methods –
- Drug sensitivity –

- Blood -VDRL
  HIV

Treatment given:

- Topical
- Oral

Follow up

ஒப்புதல் படிவம்

1. ஆராய்ச்சியின் தலைப்பு :

   படிவம் எண் :

   பங்கு பெறுபவரின் பெயர் :

   வயது / பிறந்த தேதி :

   முகவரி :

1. எனக்கு (பங்கு பெறுவோர்) இந்த ஆராய்ச்சியின் முழு விவரங்களும் தெரிவிக்கப்பட்டது.

2. என்னுடைய பங்களிப்பினை எந்த ஒரு சூழலிலும், எவ்வித காரணமுமின்றி விலக்கிக் கொள்ளவும் முழு உரிமை அளிக்கப்பட்டுள்ளது என்பதையும் அறிவேன்.

3. இந்த ஆராய்ச்சியின் முடிவையோ, என்னைப் பற்றி தகவல்களோ வேறு எவருக்கும் தெரிவிக்கப்பட மாட்டாது எனவும் உறுதி அளிக்கப்பட்டது என்பதையும் அறிவேன்.

4. நான் இந்த ஆராய்ச்சியின் தகவல்களை மேற்கூரிய ஆராய்ச்சி படிப்புக்கு பயன்படுத்திக் கொள்ள எனது முழு சம்மதத்தை தெரிவிக்கிறேன்.

5. மேற்கண்ட அனைத்து ஒப்புதலுடன் என் முழு மனதுடன் இந்த ஆராய்ச்சியில் பங்கேற்கிறேன்.

பங்கு பெறுவோரின் கையொப்பம் : தேதி :

பங்கு பெறுவோரின் பெயர் :

மருத்துவரின் கையொப்பம் : தேதி :

மருத்துவரின் பெயர் :

சாட்சிகள் : தேதி :

****

INFORMED CONSENT FORM

Study Title _____

Study Number _____

Subject's Full Name _____

Date of Birth/Age _____

Address _____

1. I confirm that I have read and understood the information sheet dated for the above study and have had the opportunity to ask questions.
   OR I have been explained the nature of the study by the Investigator and had the opportunity to ask questions
2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the clinical trial/project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s)
5. I agree to take part in the above study

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative: _____

Signatory's Name _____ Date _____

Signature of the Investigator _____ Date _____

Study Investigator's Name _____

Signature of the Witness _____ Date _____

Name of the Witness

ABBREVIATIONS IN MASTER CHART

HIV	-	Human Immune Deficiency Virus
BV	-	Bacterial Vaginosis
TV	-	Trichomonas Trichomonas Vaginalis
W	-	Genital Warts
HSV	-	Herpes Genitalis
OC	-	Oral Candidiasis
DM	-	Diabetes Mellitus
PID	-	Pelvic Inflammatory Disease
AB	-	Antibiotics
IUCD	-	Intra Uterine Contraceptive Devices
IMDr	-	Immunosuppressive Drug Intake
VVC	-	Vulvovaginal Candidiasis
RVVC	-	Recurrent Vulvovaginal Candidiasis
YES	-	Culture Positives
CN	-	Culture Negatives
S	-	Sensitive
R	-	Resistant

S.No.	Name	Age	Clinical Features								Pre Disposing factors	CO-Infections	RVVC	C Albicans	Non C Albicans			Drug Susceptibility						
			Itch	Scanty Discharge	Profuse Discharge	Erythema / Infl	Erosion	Soddening	Maceration	Satellite Pustules					C.glabrata	C.tropicalis	C.krusei	Fluconazole	Itraconazole	Voriconazole	Nystatin	Clotrimazole	Miconazole	Ketoconazole
1	Muthathal	60	+	-	+	+	+	+	-	-			*Yes*			*Yes*		*S*	*S*	*S*	*S*	*S*	*R*	*R*
2	Vadivoo	32	+	+	-	-	-	-	-	-				*CN*										
3	Kamsala Devi	28	+	+	-	-	-	-	-	-				*CN*										
4	Iyyammal	21	+	-	+	-	-	-	-	-				*CN*										
5	Devi	32	+	+	-	-	-	-	-	-				*CN*										
6	Selvi. N	40	+	-	+	-	-	-	-	-	HIV			*CN*										
7	Ramalakshmi	37	+	-	+	-	-	-	-	-	HIV	BV		*CN*										
8	Ameena	46	+	-	+	-	-	-	-	-				*CN*										
9	Mariammal. P	30	+	-	+	-	-	-	-	-	HIV			*CN*										
10	Subbulakshmi	32	+	-	+	-	-	-	-	-	HIV			*CN*										
11	Valarmathi	25	+	-	+	-	-	-	-	-		**TV**	**Yes**	**Yes**				**S**	**S**	**S**	**S**	**S**	**R**	**R**
12	Mary	38	+	-	+	-	-	-	-	-	HIV			**Yes**				**S**	**S**	**S**	**S**	**R**	**R**	**R**
13	Vijila	33	+	-	+	-	-	-	-	-				**Yes**				**S**	**S**	**R**	**S**	**R**	**R**	**R**
14	Muthuselvi	36	+	-	+	-	-	-	-	-	HIV													
15	Mary Vasantha	45	+	-	+	-	+	+	-	-	DM	WART	**Yes**					**S**	**S**	**S**	**S**	**S**	***R***	***R***
16	Mala	36	+	-	+	-	-	-	-	-	HIV/OC		**Yes**	**Yes**				**S**	**R**	**S**	**S**	**S**	**S**	**S**
17	Palani Ammal	50	+	-	+	-	-	-	-	-	HIV	BV		**CN**										
18	Thilagavathy	35	+	-	+	-	-	-	-	-	HIV		**Yes**	**Yes**				**S**	**R**	**R**	**S**	**R**	**S**	**S**
19	Sermakani. Sekar	47	+	-	+	-	-	-	-	-	HIV	HSV	**Yes**	**Yes**				**S**	**S**	**S**	**S**	**S**	**R**	**R**
20	petchiammal	53	+	-	+	+	-	-	-	-	DM	TV/W	**Yes**	**CN**				**S**	**S**	**R**	**S**	**S**	**R**	**R**
21	Shanmugathai	45	+	-	+	-	-	-	-	-				**Yes**				**S**	**R**	**R**	**S**	**S**	**S**	**R**
22	Vijayalakshmi	51	+	-	+	-	-	-	-	-	DM			**CN**										
23	Bagiyalakshmi	27	+	-	+	-	-	-	-	-				**Yes**				**S**	**S**	**R**	**S**	**S**	**S**	**R**
24	Thamarai Selvi	40	+	-	+	-	-	+	-	-	DM/AB		**Yes**	**CN**				**S**	**S**	**S**	**R**	**S**	**R**	**R**
25	Jebaral	45	+	-	+	-	-	-	-	-				**Yes**				**S**	**S**	**S**	**S**	**S**	**R**	**R**
26	Vasantha	30	+	-	+	-	-	-	-	-		TV		**Yes**				**S**	**S**	**S**	**S**	**S**	**R**	**R**
27	Mallika	26	+	-	+	-	-	-	-	-	HIV			**Yes**				**S**	**S**	**R**	**S**	**S**	**R**	**R**
28	Latha	41	+	-	+	-	-	-	-	-	HIV			**CN**				**R**	**S**	**S**	**S**	**S**	**S**	**R**
29	Nithyalaksmi	28	+	-	+	-	-	-	-	-	HIV/OC			**Yes**				**R**	**R**	**R**	**S**	**S**	**S**	**R**

S.No.	Name	Age	Clinical Features								Pre Disposing factors	CO-Infections	RVVC	C Albicans	Non C Albicans			Drug Susceptibility						
			Itch	Scanty Discharge	Profuse Discharge	Erythema / Infl	Erosion	Soddening	Maceration	Satellite Pustules					C.glabrata	C.tropicalis	C.krusei	Fluconazole	Itraconazole	Voriconazole	Nystatin	Clotrimazole	Miconazole	Ketoconazole
30	Amutha	27	+	-	+	-	-	-	-	-	HIV			Yes				R	S	S	R	S	S	R
31	Mary Stella	34	+	-	+	-	-	-	-	-	HIV		Yes	CN										
32	Boomari	42	+	-	+	-	-	-	-	-	HIV/OC			CN										
33	Shanmuga Sundari	51	+	-	+	+	-	-	+	-	DM	HSV		Yes				S	S	S	S	R	R	R
34	Muneeswari	23	+	-	+	-	-	-	-	-	HIV		Yes	CN										
35	Rajalakshmi	45	+	+	-	-	-	-	-	-	HIV			CN										
36	Sermakani. V	29	+	+	-	-	-	-	-	-	HIV			Yes				S	S	S	R	S	S	R
37	Mariammal. M	38	+	+	-	-	-	-	-	-				CN										
38	Priya	22	+	-	+	+	-	-	-	-				Yes				R	R	S	S	S	S	R
39	Mari Selvi	31	+	+	-	+	-	-	-	-				Yes				S	S	S	S	S	R	R
40	Kala	33	+	-	+	+	-	-	+	+	HIV		Yes	Yes				S	S	S	S	S	R	R
41	Katharpath	35	+	-	+	-	-	-	-	-	IM Dr			Yes				S	S	S	S	S	R	R
42	petchiamml	37	+	+	-	-	-	-	-	-	HIV			CN										
43	Selvi Durai	35	+	-	+	-	-	-	-	-	HIV			Yes				S	S	R	S	S	R	R
44	Shanthi Mariappan	42	+	-	+	-	-	-	-	-				Yes				S	R	R	S	R	R	S
45	Muthu Kalaiyarasi	32	+	-	+	-	-	-	-	-			Yes	CN										
46	Sri Seethalakshmi	29	+	-	+	-	-	-	-	-							Yes	R	S	S	S	S	S	R
47	Vasanthi	51	+	-	+	-	-	-	-	-	IM Dr			Yes				S	S	S	S	R	R	R
48	Nagomi	33	+	+	-	-	-	-	-	-	HIV/OC			Yes				S	S	S	S	S	S	S
49	Shanthi Thangaraj	34	+	-	+	-	-	-	-	-	DM			Yes				S	S	S	S	S	S	S
50	Shenbagavalli	37	+	-	+	-	-	-	-	-	HIV/OC/AB		Yes	Yes				S	S	S	S	S	R	R
51	Mariammal Serva	38	+	-	+	-	-	-	-	-				CN										
52	Ravathi	26	+	-	+	-	-	-	-	-	HIV			CN										
53	Roselin	29	+	+	-	-	-	-	-	-	HIV/OC/AB			Yes				S	R	S	S	R	R	S
54	lakshmi	45	+	+	-	-	-	-	-	-	AB	BV		CN										
55	Vellairose	25	+	-	+	-	-	-	+	-	IUCD			Yes				R	R	R	S	S	S	R
56	Nabisha	29	+	-	+	+	-	-	+	-	AB				Yes			R	S	R	S	R	R	R
57	Jyothi	40	+	-	+	-	-	-	-	-	HIV			CN										
58	Devi	28	+	-	+	-	-	-	-	-				CN										

S.No.	Name	Age	Clinical Features								Pre Disposing factors	CO-Infections	RVVC	C Albicans	Non C Albicans			Drug Susceptibility						
			Itch	Scanty Discharge	Profuse Discharge	Erythema / Infl	Erosion	Soddening	Maceration	Satellite Pustules					C.glabrata	C.tropicalis	C.krusei	Fluconazole	Itraconazole	Voriconazole	Nystatin	Clotrimazole	Miconazole	Ketoconazole
59	Mari	33	+	-	+	-	-	-	-	-	HIV			Yes				S	S	S	S	S	S	S
60	Velammal	34	+	+	-	+	-	-	-	-	DM			Yes				S	R	S	S	S	S	S
61	Rani	20	+	+	-	-	-	-	-	-	IUCD		Yes		Yes			S	S	S	S	S	S	S
62	Sumathi	30	+	-	+	-	-	-	-	-	HIV			CN										
63	Pappa	37	+	-	+	-	-	-	+	-	HIV/AB	WART		CN										
64	Mariselvam	23	+	-	+	-	-	-	-	-				CN										
65	Packiam	35	+	-	+	-	-	-	+	-	HIV			CN										
66	Velthai	37	+	-	+	-	-	-	-	-	HIV/OC			Yes				S	S	S	S	S	S	S
67	Arokia Selvi	46	+	+	-	-	-	-	-	-		PID		CN										
68	mari	35	+	+	-	-	-	-	-	-	HIV			Yes				S	S	S	S	S	S	S
69	Mary Jesintha	42	+	+	-	-	-	-	-	-				Yes				S	S	S	S	S	S	S
70	Arasammal	43	+	-	+	-	-	-	-	-	HIV/OC			Yes				S	S	S	S	S	S	S
71	Indira	36	+	-	+	-	-	-	-	-		BV				Yes		S	S	S	S	S	S	S
72	Jebaseela	21	+	-	+	-	-	-	-	-	HIV					Yes		S	S	S	S	S	S	S
73	Malar Vizhi	31	+	-	+	-	-	-	-	-	HIV			CN				R	S	R	S	R	S	R
74	Selvi. S	36	+	-	+	-	-	-	-	-	HIV	HSV		CN										
75	Kalyani	38	+	-	+	-	-	-	-	-				CN										
76	Backya Lakshmi	33	+	-	+	-	-	-	-	-	HIV			Yes				S	S	S	S	S	S	S
77	Sindhu kumari	28	+	-	+	-	-	-	-	-					Yes			S	R	S	S	S	S	S
78	Epsibulah	35	+	-	+	-	-	-	-	-	AB						Yes	S	R	S	S	S	S	S
79	Ganga Lakshmi	44	+	-	+	-	-	-	-	-	HIV	BV			Yes			S	R	S	S	S	S	S
80	Mariammal. C	30	+	+	-	-	-	-	-	-	AB			Yes				S	S	S	R	S	S	R