

CHAPTER ONE

1. INTRODUCTION

1.1. Introduction

Vulvovaginal Candidiasis (VVC) is described as consisting of a white “cottage cheese” discharge with associated vulval and vaginal inflammation (Zhou *et al.*, 2009).

The vagina could be infected by a variety of pathogens including bacteria, fungi, viruses, and parasites. Vaginal complaints such as Bacterial Vaginosis (BV), candidiasis, trichomoniasis, and *Chlamydia trachomatis* infections are common among women of reproductive age, with high incidences during pregnancy (Abdelaziz *et al.*, 2014).

Microbial infections of the vagina (vaginosis and vaginitis) among pregnant women are serious problems because they can lead to serious medical complications such as preterm labor, amniotic fluid infection, premature rupture of the fetal membranes, and low birth weight of the neonate (LBW), leading to high prenatal mortality. However, proper identification and treatment will reduce the risk of preterm birth and its consequences (Abdelaziz *et al.*, 2014., Rasti *et al.*, 2013).

Vulvovaginal candidiasis (moniliasis or thrush) is a commonly reported gynecological condition and frequently distressing infection for many pregnant women (Kamath *et al.*, 2014). Which may cause systemic infections in neonate (Rasti *et al.*, 2013). And is diagnosed in a large proportion of women of different age groups, regardless of their sexual activities presenting to medical facilities with a complaint of abnormal vaginal discharge (Rathod and Buffler, 2014., Oyewole *et al.*, 2013).

Numerous studies around the world showed that *Candida albicans* is responsible for the largest number of symptomatic episodes of vaginal candidiasis. Non-*albicans* species are most commonly represented by *C. tropicalis*, *C. glabrata*, and *C. krusei*. Accurate species identification is important for the treatment of the *Candida* infections, as the non-*albicans* species of *Candida* continue to be increasingly documented (Kazemi *et al.*, 2013).

While not a cause of mortality, the morbidity associated with vulvovaginal candidiasis make it a major cause of mental distress and economic costs. Though there are well-recognized limitations of the existing epidemiologic data for vulvovaginal candidiasis (Rathod and Buffler, 2014).

For unknown reasons, they are more prevalent in women in sub-Saharan Africa and other low-income countries than in women in developed countries, affecting up to 55% of women in some studies (Abdelaziz *et al.*, 2014).

In developing countries, there is scanty data on vaginal candidiasis in pregnant women and the distribution of the vaginal *Candida* species (Nelson *et al.*, 2013). In Sudan, there is little data about vaginal infections in pregnant women; a cross-sectional study of pregnant women was conducted at Omdurman Maternity Hospital, Khartoum, Sudan, found *Candida albicans* was detected in (16.6%). Higher infection rates were recorded among subjects in the third trimester (71.6%) than in the second trimester of gestation (28.4%) (Abdelaziz *et al.*, 2014). Also another study conducted to determine the prevalence of bacterial vaginosis and vaginal candidiasis in the 100 pregnant women was to 14%, 73% respectively. *Gardnerella vaginalis* was isolated in 14 (14%) samples, *Candida albicans* in 73 (73%) samples, combined infection with *Gardnerella vaginalis* and *Candida albicans* was detected in 3 (3%) samples (Ahmed *et al.*, 2014).

1.2. Rationale

Vaginal candidiasis in untreated pregnant women can lead to transmission to their infant (such as cutaneous candidiasis of neonates) and may be associated with sterility and sub fertility as the complication of Pelvic Inflammatory Disease (PID) (Parveen *et al.*, 2008), because of this accurate and prompt diagnosis is mandatory. Also the laboratory can help the clinician to distinguish between different types of infections.

Increased awareness about mycosis and partial availability of resources were a motive to start establishing a specific technique in diagnosis of fungal vaginitis.

1.3. Objectives

This study designed to fulfill the following objectives:

1.3.1. General objective

To detect the frequency of vaginal candidiasis among pregnant women with whitish vaginal discharge attending Bashaier Teaching Hospital in period from March to June 2014.

1.3.2. Specific objectives

- I. To isolate and identify *Candida* species.
- II. To establish the frequency of *Candida albicans* among pregnant women.
- III. To determine the association of vaginal candidiasis with age, trimester, gravidity and antifungal treatment.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Vaginal Candidiasis (VC)

2.1.1. Anatomy of the female genital tract

The female reproductive system consists of the paired ovaries and fallopian tubes, the single uterus and vagina, and the external genital structures (Scanlon and Sanders, 2007).

A normal function of the vaginal walls and the cervix is to produce secretions. The secretions can sometimes be noticed outside of the vagina (this is referred to as 'normal vaginal secretions and discharge change from time to time; sometimes clear, almost like water, and at other times, mucousy and whitish in color, sometimes scant and, at other times, larger in amount. These are normal variations. Normal vaginal secretions and discharge may vary from one woman to another depending on: the stage of the menstrual cycle, menopause, whether a woman is taking contraceptives or hormone replacement medications, whether the woman is pregnant or not and state of sexual arousal (Berkow *et al.*, 2001).

2.1.2. Definition of VC

Wilkinson described vaginal candidiasis for the first time in 1849 (Kamath *et al.*, 2014).

Vulvovaginal Candidiasis (VVC) is a fungal infection caused by overgrowth of *Candida* species affecting the genital tract as opportunistic pathogen (Oyewole *et al.*, 2013). Vaginal candidiasis (VC) is one of the most common type of vaginal infections (vaginitis) in women, in the fertile period, and also the most frequent and most important fungal disease of vaginal content (Babić and Hukić, 2010).

Candida is the agent most frequently implicated in the invasive vaginal candidiasis. The most common *Candida* species causing vaginal candidiasis is primarily *Candida albicans* (Oyewole *et al.*, 2013) and responsible for 70-90% of all vaginal candidiasis (Imran and Al-Shukry, 2014., Rad *et al.*, 2013). The remainder are non-albicans *Candida* species, of which the most common is *Candida glabrata* is responsible for 14% of infections in immune competent women (Imran and Al-Shukry, 2014), followed by *Candida tropicalis* and *Candida parapsilosis* (Oyewole *et al.*, 2013., Nelson *et al.*, 2013) and occasionally, *C. krusei*, *C. pseudotropicalis*, and *C. dubliniensis* (Oyewole *et al.*, 2013).

Vulvovaginal Candidiasis (VVC) can be classified as either uncomplicated (as in ~ 90% of cases) or complicated (~10% of cases) on the basis of clinical presentation, microbiological findings, host factors, and response to therapy. Complicated VVC is defined as severe or recurrent disease, infection due to *Candida* species other than *C. albicans*, and or VVC in an abnormal host (Pappas *et al.*, 2009). The British Association for Sexual Health and HIV (BASHH) states that 10-20 percent of patients with recurrent VVC have non-albicans *Candida* infections. Vaginitis caused by non-albicans species is clinically indistinguishable from that caused *C. albicans*, and importantly, non-albicans species can be more resistant to treatment (Ramsay *et al.*, 2009).

Nearly 75% of women have at least one episode of genital yeast infection in their reproductive years and 10-20% of women have asymptomatic vaginal colonization with *Candida* species. Pregnant women with diabetes are more susceptible and vaginal mycosis is four times higher in them. Although recurrent episodes of vaginal candidiasis are common, a marked proportion of women with chronic and recurrent infection may present first time during pregnancy (Parveen *et al.*, 2008).

Before proceeding with empirical antifungal therapy, diagnosis should be confirmed by a wet mount preparation with use of saline and 10% potassium

hydroxide to demonstrate the presence of yeast or hyphae. In addition, VVC is associated with normal pH (< 4.5). For those with negative wet mount findings, vaginal cultures for *Candida* should be obtained (Pappas *et al.*, 2009).

2.1.3. Signs and Symptoms

Vulvovaginal candidiasis (VVC) is a gynecologic disorder. A diagnosis of *Candida* VVC can usually be made clinically when a woman complains of vulval and or vaginal pruritus, irritation, burning , vaginal soreness (Oyewole *et al.*, 2013), itching, pain (Imran and Al-Shukry, 2014), external dysuria, and dyspareunia (Pappas *et al.*, 2009).

Signs include vulval and vaginal edema, erythema (Patel *et al.*, 2004), excoriation, fissures, and a white, thick, curd-like vaginal discharge (non offensive) (Aslam *et al.*, 2008). Unfortunately, these symptoms and signs are nonspecific and can be the result of a variety of infectious and noninfectious etiologies (Pappas *et al.*, 2009).

2.1.4. Predisposing factors

When *Candida* species e.g. *C. albicans* outgrows other friendly organisms in the genital tract, it disrupts the balance in the host as a result of response to the changes in the environment and becomes disease-causing pathogen. This immune imbalance is caused by a number of factors, such as excess stress, allergies, steroids, and nutrient deficiency (Oyewole *et al.*, 2013).

Several additional factors like pregnancy (30-40%), use of high estrogen content oral contraceptives only partially explain recurrent vulvovaginal candidiasis (RVVC), attendance at sexually transmitted diseases clinics (Babić and Hukić, 2010), diabetes mellitus (DM) (Oyewole *et al.*, 2013) and age (Babić and Hukić, 2010). Reproductive hormones, also predispose women to acute and chronic VVC (Aslam *et al.*, 2008), genetic factors (Zhou *et al.*, 2009), IUCDs (intrauterine contraceptive device), use of vaginal douches and unsanitary living conditions (Parveen *et al.*, 2008).

The most frequently implicated risk factors include the use of broad-spectrum antibacterial agents has been suggested as a risk factor for both acute and recurrent VVC (Patel *et al.*, 2004), use of central venous catheters, receipt of parenteral nutrition, receipt of renal replacement therapy by patients in ICUs, neutropenia, use of implantable prosthetic devices (Pappas *et al.*, 2009). HIV infection and being immunocompromised (Imran and Al-Shukry, 2014).

Other predisposing conditions are malignant diseases such as lymphomas or leukemias, aplastic anemia, drug treatments (corticosteroids, antidepressants, antineoplastic drugs and immunosuppressants), hyposialia (produced by disorders such as Sjögren's disease, drugs or radiotherapy), and terminal or end-stage systemic diseases (Castellote and Soriano, 2013., Kamath *et al.*, 2013).

Most popular literature focuses on behavioral factors, but there are relatively few studies of these factors, particularly for recurrent infections. Dietary factors, clothing habits, personal hygiene, and sexual practices have been studied as risk factors for recurrence. However, the results have not been conclusive (Patel *et al.*, 2004).

Pregnancy is a physiological state, which produces several normal and expected changes in all the maternal organ systems (Kamath *et al.*, 2013). Vaginal candidiasis has been related to emotional stress and suppression of immune system which step up the risk of *Candida* species over growth and become pathogenic (Zhou *et al.*, 2009).

Pregnancy induced hormonal modifications. The increased secretion of reproductive hormones during pregnancy altered the vaginal context and made *Candida* more likely to grow beyond acceptable boundaries and favors the formation of infection (Babić and Hukić, 2010., Nelson *et al.*, 2013). High levels of estrogen provide an increased amount of glycogen in the vagina, furthermore providing a good source of carbon needed for *Candida* growth and their

germination. These hormones accelerate the formation of yeast pseudopyphae (Babić and Hukić, 2010).

The condition is rare before puberty but by the age of 25, nearly 50% of all women will have had at least one clinician diagnosed episode of VVC. The condition is less common in postmenopausal women (Moallaei *et al.*, 2011), due to hormonal dependence of vaginal candidiasis (Babić and Hukić, 2010).

Other risk factors are associated with the eating habits of pregnant women of sugar rich containing food. The sugar increase ever more the threat of yeast infections powered by these sugary environments (Zhou *et al.*, 2009).

2.1.5. *Candida*

2.1.5.1. Classification

Candidiasis is an infection caused by a yeast species of the genus *Candida*, which belongs to the Kingdom: Fungi, phylum: Ascomycota, subphylum: Saccharomycotina, class: Saccharomycetes, order: Saccharomycetales, family: Saccharomycetaceae, genus: *Candida* (Hajjeh *et al.*, 2004). About 20 species are known to cause infections in humans (Moris *et al.*, 2008). Includes the species *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. guilliermondii*, *C. lusitaniae*, and *C. krusei*, *C. dubliniensis*, *C. pelliculosa*, *C. kefyr*, *C. norvegensis*, *C. haemulonii*, and *Saccharomyces cerevisiae* (Aittakorpi *et al.*, 2012).

2.1.5.2. General characteristics

Candida yeasts have globose, ellipsoidal, cylindroidal, or elongate, occasionally ogival, triangular or lunate cells (Moris *et al.*, 2008). Measuring 3-30 µm in diameter (Castellote and Soriano, 2013).

Candida albicans exists naturally as diploid yeast and until recently was thought to be asexual, as no direct observations of mating or meiosis had been reported (Carlisle and Kadosh, 2013). *C. albicans* exhibits the ability to grow as either a

yeast or a mycelial form in response to different environmental factors (Kim *et al.*, 2002).

It reproduces asexually through a budding process in which protoplasmic protrusions or buds (blastoconidia) emerge from the mother cell and grow until they finally detach to form a new cell. The daughter cells occasionally do not detach and form chains of cells called pseudohyphae, which can be mistaken for hyphae (Castellote and Soriano, 2013).

The three primary morphological forms, blastospores, pseudohyphae and hyphae, are all found in infected tissues, and work to date indicates that the transition between these forms is critical for pathogenesis (Andrew *et al.*, 2003).

2.1.5.3. Habitat

Candida albicans is a natural component of the human microbiome. This fungus asymptotically colonizes many areas of the human body, especially the skin, gastrointestinal (oral cavity or mouth of 25–50% healthy individuals, intestines) and genitourinary tracts (mainly vagina) of healthy individuals (Fox and Nobile, 2012) (Imran and Al-Shukry, 2014). *Candida* species are confined to human and warm-blooded animal reservoirs; however, they can also be recovered from soil, food, water and, sometimes, air (Moris *et al.*, 2008).

2.1.5.4. Virulence factors

There are differences in pathogenicity among *Candida* spp. isolates. Some properties related to *Candida albicans* cells give them the capacity to cause disease. Adherence to cell surface, germ tube formation with consequent development of the filamentous form, phenotypic variability, and production of toxins and extracellular enzymes constitute important factors for the emergence of infections by *Candida*. The enzymes produced then, especially proteinase and phospholipase, allowed the yeast penetration into the cells, inducing inflammatory response with injury of adjacent tissues (Moris *et al.*, 2008).

To date, most studies of *C. albicans* have been performed in suspension cultures; however, the medical impact of *C. albicans* (like that of many other microorganisms) depends on its ability to form surface-associated communities called biofilms (Fox and Nobile, 2012).

2.1.6. Pathogenesis

Vaginal candidiasis is one of the mucous opportunistic infections. It is still not definitively determined what exactly leads to disruption of the balance and origin of infection. Vaginal candidiasis occurs in the presence of factors that increase the virulence of *Candida*, and as a result of the reduction in local defense mechanisms. The exact mechanism by which *Candida* infection occurs is not clear. It is possible that there are multiple mechanisms by which *Candida* can cause cell damage and lead to direct invasion of the infection hyphae in epithelial tissues. During vaginal candidosis, vagina is the normal pH range (pH 4.0 – 4.5), as opposed to mixed infections (bacterial, *Trichomonas*), where pH rises to levels greater than (4.7) (Babić and Hukić, 2010).

There is a balance between *Candida*, normal bacterial flora, and immune defense mechanisms. When this balance is disturbed, colonization is replaced by infection (Babić and Hukić, 2010). This infection progresses as colonization, superficial infection and hematogenous dissemination to different organ (Oyewole *et al.*, 2013).

The formation of germinal tubes and the presence of certain glycoproteins such as mannose and glucose in the fungal wall facilitate adherence to cell membranes and receptors. At the same time, the presence of germinal tubes and the production of phospholipase C also facilitate fungal invasion. Some components of this yeast-like alter the host defense mechanisms (e.g., inhibiting phagocytosis). On the other hand, certain polysaccharides in the fungal wall induce suppressor T lymphocytes, and mannan found in the wall can interfere with antigen presentation, inhibiting the

immune defense response. Tissue damage results from direct action of the microorganism but also from the host defenses developed against tissue invasion, including IgE-mediated immune allergic reactions against the fungal antigens and delayed hypersensitivity reactions. Thus, in order for *Candida* to produce infection, it must colonize, invade and multiply (Castellote and Soriano, 2013).

The mechanisms by which pregnancy encourages *Candida* colonization are complex. During pregnancy, levels of both progesterone and estrogen hormones are elevated. Progesterone has suppressive effects on the anti-*Candida* activity of neutrophils, while estrogen have been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candidia albicans* and also decreases immunoglobins in vaginal secretions resulting in increased vulnerability of pregnant women to vaginal candidiasis (Aslam *et al.*, 2008).

Vaginal secretions during pregnancy fall from a pH of greater than 7 (an alkaline pH) to 4 or 5 (an acid pH). This occurs because of the action of *Lactobacillus acidophilus*, bacteria that grow freely in the increased glycogen environment, and by so doing increase the lactic acid content of secretions. This changing acid content helps to make the vagina resistant to bacterial invasion for the length of the pregnancy. This change in pH also unfortunately, favors the growth of *Candida albicans* (Kamath *et al.*, 2013).

Women with VVC did not have reduced numbers of *Lactobacillus* species. Further, there is no consensus on whether H₂O₂-producing lactobacilli in the vaginal microbial communities protect their hosts from VVC. It has also been observed that the use of antibiotics can lead to yeast infections, perhaps by killing or inhibiting bacterial populations that have antimycotic properties. This suggests that normal vaginal microbiota may have an important role in restricting yeast infections. Trials to evaluate the use of oral or vaginal probiotics containing

Lactobacillus spp. to prevent postantibiotic VVC have been limited and have yielded inconsistent results (Zhou *et al.*, 2009).

The high incidence and healthcare costs associated with treatment of VVC highlight the need for understanding the pathogenesis of the infections and host defense mechanisms so that effective strategies can be developed to control and prevent this disease. Relatively little is known about possible associations between the bacterial species found in the vagina and colonization by *Candida* species. While it is commonly thought that the normal vaginal microbiota plays an important role in the prevention of vaginal infections and transmission of pathogens responsible for sexually transmitted diseases (STDs), there is no consensus about the impact of vaginal bacteria on control of VVC and RVVC (Zhou *et al.*, 2009).

2.1.7. Immunity

The normal flora (bacteria) of the vagina creates an acidic pH that helps inhibit the growth of pathogens (Scanlon and Sanders, 2007). More than 50 different species of bacteria may live in a woman's vagina, with lactobacilli being the predominant microorganism found in healthy women estimated 96% and its concentrations is 10⁵ to 10⁸ / ml. The other microorganisms which present in vagina as normal flora are facultative organisms which include Diphtheroid, *Streptococci*, *Escherichia coli*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and anaerobic organisms such as *Peptostreptococci*, *Bacteroid* and *Fusobacterium* (Berkow *et al.*, 2001). Disturbance of the normal vaginal pH and estrogen levels can alter the vaginal flora, leading to overgrowth of pathogens. Factors that alter vaginal environment include feminine hygiene products, contraceptives, vaginal medications, antibiotics, STDs, sexual intercourse, and stress (Berkow *et al.*, 2001).

Mechanical barriers, inflammatory cells and cellular immunity restrict *Candida* spp. to non-sterile superficial sites. Besides, the resident bacterial microbiota

generally limits the number of fungal cells, blocks their adhesion to epithelial cells, competes with them for nutrients, and prevents the fungus conversion into its most invasive form; the filamentous form (Ventolini, 2013). *Candida* antigens stimulate specific cellular and humoral immune response. With regard to innate immunity, macrophages and neutrophils seem not to have a relevant role (Moris *et al.*, 2008). The Th1 branch of cell-mediated immune response is considered fundamental for such defense. Thus, epithelial cells may represent an important mechanism of innate resistance against mucous candidiasis as they inhibit fungal cell growth, which is attributed to a portion constituted of carbohydrates (still not determined but existent) at the cells surface (Moris *et al.*, 2008).

2.1.8. Incidence and prevalence

Numerous studies around the world show that *Candida albicans* is responsible for the largest number of symptomatic episodes of vaginal candidiasis. Percentage of infection that causes *C. albicans* was high in the past decades, and varied from 85 to 90%. Non-albicans species are most commonly represented by *C. glabrata*, and *C. tropicalis*. During the 1970^s, the incidence of non-albicans species in the United States amounted to 5- 10% (Babić and Hukić, 2010).

The incidence of vaginal candidiasis is almost doubled (particularly in the second and third trimester) among pregnant women, due to high production or changes in the levels of sex hormones and deposition of glycogen in the vagina during pregnancy (Oyewole *et al.*, 2013). Also multigravida suffers significantly more than primigravida. Moreover, a significant proportion of women with chronic or recurrent candidiasis first present with this infection while pregnant (Aslam *et al.*, 2008). The impaired cellular immunity" and decreased *Candidacidal* effects of leucocytes associated with pregnancy may also contribute to the increased incidence (Aslam *et al.*, 2008). Though there are well-recognized limitations of the existing epidemiologic data for vulvovaginal candidiasis, commonly reports that

approximately: 75% of women during the fertile period have at least one episode of VVC (Rathod and Buffler, 2014., Babić and Hukić, 2010., Oyewole *et al.*, 2013). Approximately 40 -50% of women have repeated infection (initially infected women will experience at least a second episode). Less than 5% of adult female population receives repeated, frequent attacks of recurrent vulvovaginal candidosis (RVVC) which is defined as four or more proven episodes of VVC in a 12 month period (≥ 4 episodes/1 year) (Rathod and Buffler, 2014., Babić and Hukić, 2010). The quality of life is greatly diminished for women who experience RVVC (Zhou *et al.*, 2009).

Point-prevalence studies indicate that *Candida* species may be isolated from the genital tract of approximately 20% (range 10 -55%) of asymptomatic, healthy women in the child-bearing age. Twenty five to 40% of women who are culture positive for *Candida* species in the vaginal area are asymptomatic carriers (Babić and Hukić, 2010).

The extent to which vulvovaginal candidiasis is a source of population-level morbidity remains uncertain (Rathod and Buffler, 2014).

2.1.9. Diagnosis of VC and identification of *Candida* species

Unfortunately, none of the clinical signs and symptoms of VVC either individually or collectively are pathognomonic of the disease. As a myriad of infectious and non infectious factors may cause identical signs and symptoms, a reliable diagnosis cannot be made on the basis of clinical evidence alone without the corroborative evidence of laboratory tests. Although culture is the most sensitive method of diagnosis of VVC, clinicians usually recommend immediate diagnosis based on Gram's stained smear (Aslam *et al.*, 2008).

There are several available procedures to identify *Candida* spp., most of which consist in the association of morphological and biochemical traits like the form and size of blastoconidia, the production of chlamydoconidia, pseudohyphae and true

hyphae, and the capacity to assimilate carbohydrate and nitrogen and to ferment different sugars in sabouraud's agar and blood agar. *Candida* spp. grows within 24-48hours at 37°C in the form of marble-white, humid, circular, convex colonies (Moris *et al.*, 2008).

True hyphae are formed from yeast-like cells through development of the germ tube. Germ tubes are formed within three hours at 37°C, in the presence of albumin, only in about 95% *C. albicans* and *C. dubliniensis* cultures. Such characteristic is used in routine laboratory for identification (Moris *et al.*, 2008).

Chlamydospore or chlamydoconidium is a resistance structure formed by thick cell wall and condensed cytoplasm. It is generally produced when the yeast is under unfavorable growth conditions. *Candia albicans* and *C. dubliniensis* produce round chlamydoconidia at intercalary or terminal position to the pseudohypha (Moris *et al.*, 2008).

Assimilation test, also called auxanogram, evaluates the capability of certain yeasts to utilize several compounds as the only source of carbon or nitrogen in the presence of oxygen. Each species has its own assimilation pattern. The capability of a yeast species to ferment certain sugars (which is determined by the fermentation test or zymogram) contributes to distinguish between species.

The systems API 20C which contain dehydrated substrates for assimilation tests, and the Vitek Yeast Biochemical Card (YBC) are examples of semi-automated methods (Moris *et al.*, 2008).

Recently, different chromogenic culture media capable of distinguishing *C. albicans* from other yeasts of clinical interest have been commercialized (Moris *et al.*, 2008).

2.1.10. Background studies

In Nigeria (2012), study was conducted to determine the prevalence of vaginal candidiasis among pregnant women. A total of sixty (60) pregnant women were examined. A total of 42 (70%) isolates were obtained which comprises five different *Candida* species, namely *Candida albicans*, *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. pseudotropicalis* with frequency of occurrence of 21 (50%), 9 (21.4%), 6 (14.3%), 5 (11.9%) and 1 (2.4%) respectively. The isolates obtained were related to number of pregnancy (gravidae), 25 (59.5%) were multi-gravidae while 17 (40.5%) were primigravidae. Pregnant women in the second trimester of pregnancy had the highest incidence of candidiasis (61%), followed by third trimester (21.4%) while the least (16.7%) was obtained in first trimester. The isolates were also related to age, the age range of 21-30 years had the highest incidence (59.5%), followed by age range of 31-40 years (31.0%) and 15-20 years had the least (9.5%) percentage of occurrence (Oyewole *et al.*, 2013).

In Maroua- Far-North Cameroon (2010), investigation was carried out at two referral hospitals, involving 211 (112 pregnant and 99 non pregnant) women of age interval 16-45 years, to estimate the prevalence of vulvovaginal candidiasis (VVC) amongst pregnant women. The results indicated that the prevalence of VVC was higher amongst pregnant (55.4%) than non-pregnant women (35.4%) (Toua *et al.*, 2013).

Another study was conducted in Kashan, Iran (2004), to find the effects of infection on the pregnancy including premature rupture of membrane (PROM), preterm delivery and low birth weight (LBW) children. The prevalence of vaginal candidiasis in pregnant women was 49 (32.7 %). 35% of the patients with preterm labor and 31.8 % with term labor were infected with *C. albicans*. Out of 12 pregnant women with PROM, four women (33.3%) were showed positive results of *C. albicans* infection, while in 138 of the mothers without PROM, *C. albicans*

was found in 45 (32.6%) ($P = 1$). Among 29 mothers who had LBW newborns, 5 (17.2%) were positive for *C. albicans* infection, while of 121 mothers with appropriate gestational age newborns, 44 (36.4 %) were showed *C. albicans* infections ($P = 0.08$). From 40 pregnant women with preterm birth, 14 (35%) was positive for *C. albicans*, but out of 110 with term labor, *Candida* was detected in 35 (31.8%) that statistically was not significant ($P = 0.7$) (Rasti *et al.*, 2013).

The prevalence of abnormal vaginal discharge in pregnancy in Maiduguri, Nigeria (2012) was 31.5%. The frequency of abnormal vaginal discharge was 183 (45.8%) among those aged 20-24 years, 291 (72.8%) in multipara, 223 (55.8%) in those with primary education and 293 (73.2%) in unemployed. Vulval pruritus 300 (75.0%) was significantly related to abnormal vaginal discharge ($P < 0.001$). The prevalence of *C. albicans* was 41%. The frequencies of vulval itching, dyspareunia and vulval excoriation among those with candidiasis were 151 (50.3%), 14 (56.0%) and 75 (75.0%) respectively ($P < 0.001$) (Ibrahim *et al.*, 2013).

In Brazil (2010), 404 women who had clinical symptoms of VVC were enrolled in this study. 70 non-pregnant women and 90 pregnant women were included. *Candida albicans* was the most prevalent, representing (72.9%) in the non-pregnant group and (92.3%) in the pregnant group. Differences in species distribution were noted between the two groups, being *C. parapsilosis* the second more prevalent species among non-pregnant women (Dias *et al.*, 2011).

In Nnewi, Nigeria (2009), from three hundred pregnant women attending pre-natal visits in three selected hospitals. Ninety patients were positive for vaginal candidiasis thus, giving a prevalence rate of (30%). The pregnant women aged 26 to 30 years recorded the highest prevalence which is statistically significant ($p < 0.05$). The women who were in their third trimester of pregnancy were mostly infected and the infection status was highly significant ($p < 0.05$) (Okonkwo and Umeanaeto, 2010).

At Isra University Hospital in Hyderabad (2005), a total of 110 pregnant women were none randomly recruited by convenient sampling. The frequency of vaginal candidiasis during pregnancy was found to be (38%), in which (27%) were symptomatic and (11%) were asymptomatic group. Increased ratio of infection was observed in multigravida and diabetic women. There was no marked difference in results with respect to age and trimester of pregnancy (Parveen *et al.*, 2008).

2.1.11. Treatment

Antifungal agents commercially available for the treatment of VVC include the following: imidazole antifungals (example, butoconazole, clotrimazole, miconazole), triazole antifungals (e.g, fluconazole, terconazole), and polyene antifungals (e.g, nystatin). These agents are available in oral and topical formulations. The topical formulations of imidazole and triazole antifungals, collectively known as azole antifungals, are considered the therapy of choice during pregnancy (Soong and Einarson, 2009).

However, the more current and most effective antifungal drugs are very expensive and out of reach for many Africans. These antifungal drugs may also be associated with some serious side effects. Research has also shown an increasing resistance of fungi to imidazole derivatives. Thus, the prescription of antifungal drugs should be hardly based on proper diagnosis and antifungal sensitivity tests. The use of medical plants, which has fewer side effects and is economically cheaper, has been recently taken into consideration (Toua *et al.*, 2013).

2.1.12. Prevention and Control

Women are unlikely to seek advice because there is a tendency to view “white discharge” as normal and also because the condition is associated with shame and guilt. Usually women complain of vaginal discharge when they think it is unusual for them or if it causes itching or discomfort (Kamath *et al.*, 2014).

To help prevent vaginal yeast infections, you can: avoid tight-fitting synthetic clothing, avoid local irritants, such as perfumed products, replace soaps with vulval water-based moisturizers- these may give symptomatic relief as dermatitis commonly co-exists; soap may also cause local irritation, change tampons and pads often during your period, wear cotton underwear and pantyhose with a cotton crotch, change out of wet swimsuits and exercise clothes as soon as you can, and keep blood sugar under control if you have diabetes (Ramsay *et al.*, 2009).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study type

This is descriptive –cross- sectional study.

3.2. Study design

Hospital and analytical study.

3.3. Study approach

Quantitative prospective study.

3.4. Study area

Facility base in Bashaier Teaching hospital, Khartoum State.

3.5. Study population and duration

Pregnant women who attend the unit of obstetric and gynecology during period of March – June 2014.

3.5.1. Inclusion criteria

Pregnant women who presented with self-reported symptoms of abnormal vaginal discharge, itching and genital burning or burning micturition.

3.5.2. Exclusion criteria

Pregnant women without abnormal vaginal discharge, catheterized patients, patients with cervical malignancies.

3.6. Sampling

3.6.1. Sample type

Non- probability sampling.

3.6.2. Sample size

One hundred pregnant women.

3.6.3. Sampling technique

Convenience.

3.7. Study variables

Screening of vaginal discharge among pregnant women (dependent variable).

Candida species, duration of pregnancy, age (by years), gravidity, antifungal treatment, medical history and laboratory data as independent variables.

3.8. Data collection

Data was collected by self interviewing questionnaire contains all study variables (appendix 1).

3.9. Ethical clearance

- Approval had been taken from the Faculty Ethical Board.
- Informed consent had been taken from all patients.
- The participants informed into their simple language about the infection, aim of the research and the benefits of the study.

3.10. Data analysis

Data was analyzed by using SPSS (Statistical Package of Social Science) soft program version 16.

3.11. Experimental Work

3.11.1. Specimen

Vaginal swab was taken from every lady.

3.11.2. Method of collection

Specimens were collected by sterile cotton tipped swabs under the gynnaecological review.

3.11.3. Macroscopic examination

The odor, consistency and color of each specimen discharge was observed and recorded.

3.11.4. Microscopic examination

Vaginal swabs were examined microscopically by 10% potassium hydroxide (KOH) wet mount and Gram's staining for the presence of budding yeast and pseudo-hyphae of *Candida* species.

3.11.4.1. Wet preparation

Swab was rolled on clean slide, 2 drops of 10% potassium hydroxide was added, and covered with cover slip, then examined under microscope using X10 and X40 for the presence of budding yeast and pseudo-hyphae of *Candida* spp (appendix 2) (Aslam *et al.*, 2008).

3.11.4.2. Gram's stain

The swab was rolled on clean slide to make smear, then smear was left to air dry, fixed with alcohol, and covered with crystal violet stain for one minute.

Then washed by tap water, and then covered with lugol's iodine one minute. Iodine was washed off, and smear was decolourized with acetone-ethanol alcohol for few seconds and washed by tap water. Safranin was added for two minutes, washed off with tap water and let to air dry and microscopically examined using oil immersion objective (X100) to observed yeast cell morphology, size, gram positive reaction and presence of pus cells, epithelial cells (appendix 3) (Bhavan *et al.*, 2010).

3.11.5. Culture

Swab was cultured on Sabouraud's Dextrose Agar (SDA) with 0.05mg/ml Gentamicin and incubated at 37 °C for 48-72 hours (appendix 4) (Bhavan *et al.*, 2010).

3.11.6. Identification of *Candida* species

3.11.6.1. Colonial Morphology

Culture was examined for pasty, creamy and smooth white colonies.

3.11.6.2. Gram's stain

Indirect Gram's stain was performed for yeast suspected colonies which revealed gram positive (G + ve) yeast cells.

3.11.6.3. Germ tube test (GTT)

Test proves yeast germination, and its characteristic for the detection of *Candida albicans*.

This is rapid test for presumptive identification of *C. albicans*. One ml of serum was added into small vitek tube by using a pasteur pipette, colony of yeast was touched by sterile wire loop and emulsified it in the serum. The tube was mixed and incubated at 37 °C for 2-4 hours but no longer, the a drop of the serum was transferred to a slide for examination, cover slip was added and examined microscopically using X40 objective.

Germ tubes are appendages half the width and 3 to 4 times the length of the yeast cell from which they arise. There is no constriction between the yeast cell and the germination tube.

Positive test: presence of short lateral filament (germ tube) for *C.albicans*,
Negative test: yeast cell only for *C. non albicans* (Bhavan *et al.*, 2010).

3.11.6.4. CHROM agar pigmentation

Chromogenic media prepared according to manufacture instruction and the organism inoculated in the media, then incubated at 37 °C for 48 hours. After that the growth of *Candida* spp observed by the change in the colour of the colonies according to the pigment, as a result of reaction between chromogenic substrate and enzymes that secreted by different *Candida* spp, allowing organisms to be identified to the species level by their color and colony characteristics.

CHROM agar *Candida* has been shown to allow differentiation of *Candida* yeast by color and morphology. The result was as the following: the product identifies *C. albicans* by growth as light to medium green and wet colonies, *C. tropicalis* by

growth as steel blue and wet colonies, *C. glabrata* dark pink and wet colonies, *C. krusei* light pink and dry colonies, and other *Candida* spp. give white color colonies (appendix 4) (Babić and Hukić, 2010).

3.11.6.5. Chlamydospore formation

By using sterile inoculating needle or loop, appropriate yeast colony was touched and immediately scraped or cut “X” through prepared corn meal agar (CMA) in the middle on one half of the agar plate, the arms of the X should be about 2 cm long. This procedure was repeated, making a duplicate “X” in the middle on the other half of the agar plate. Using sterile forceps, sterile cover slip was centered over the cross of one of the “X” patterns. Plate was inverted and incubated up to 3 days (72 hours) at $25 \pm 2^{\circ}\text{C}$. Plates were examined daily for the development of chlamydospores with the aid of dissecting or stage microscope. The “X” without cover slip serves as a growth control. The result seen by microscopic examination of the yeast under the cover slip revealed chlamydospore that appear as terminal double walled spheres on the pseudohyphae which indicated positive result (appendix 4) (Bhavan *et al.*, 2010).

3.11.6.6. Zymogram (Carbohydrate fermentation tests)

Fermentative yeasts recovered from clinical specimens produce carbon dioxide and alcohol. Production of gas rather than a pH shift is indicative of fermentation. Dextrose, maltose, sucrose, lactose, galactose and trehalose were used in the test. The 5 ml of carbohydrate (pH 7.4) containing 1 % peptone, 1 % sugar, 0.3 % beef extract and 0.5 % NaCl, 0.2 % Andrad’s in distilled water medium was dispensed in sterilized Durham tube and 0.2 ml of saline suspension of the test organism was added and incubated at 37°C for 48-27 hours up to 10 days and the fermentation pattern was read from the table (Table 1) (Bhavan *et al.*, 2010).

CHAPTER FOUR

4. RESULTS

A total number of 100 pregnant women attending to Bashaier Teaching Hospital during the period from March to June 2014 were enrolled in this study to detect the frequency and etiology of vaginal candidiasis (VC), their age ranged from 18 to 41 years with a mean of 29.6 years.

All pregnant women had characteristic symptoms of vaginal candidiasis. Vaginal swabs were collected and screened for etiology of VC.

The result revealed that, 48 (48%) samples were positive for VC, while 52 (52%) samples were negative (Figure 1).

The most frequently encountered species was *Candida albicans* 28 (58%), followed by *C. parapsilosis* 10 (21%), *C. guilliermondii* 8 (17%) and *C. glabrata* 2 (4%) (Figure 2).

According to duration of pregnancy, 24 (50%) of infected pregnant women were in third trimester, 16 (33%), 8 (17%) in first and second trimester respectively. Statistically there was significant association (p value= 0.001) between VC and duration of pregnancy (Table 2).

Regarding gravidity, most of pregnant women were multigravida (60 (60%)) and 40 (40%) primigravida, the result revealed that multigravida were more infected than primigravida, 32 (67%) versus 16 (33%). Statistical analysis showed that there is no significant association (p value= 0.191) between VC and gravidity (Table 3).

According to age group, 24 (50%) of infected pregnant women their ages were between 18-25 years. Statistical analysis showed that there is no significant association (P value= 0.142) between VC and age (Table 4).

According to antifungal treatment consumption, 16 (16%) of pregnant women were on antifungal treatment and 84 (84%) were not, the result revealed that there is significant association (p value= 0.018) between VC and antifungal treatment, 12 (25%) of pregnant women who use antifungal were infected, and 36 (75%) of pregnant women who did not use it were infected (Table 5).

Regarding to medical history of disease (VC), 14 (29%) of pregnant women have past infection; *C. albicans* is the most causative agent for all cases.

Table 1. Different reactions of all tests performed for all *Candida* spp.

Test	<i>C. albicans</i>	<i>C. parapsilosis</i>	<i>C. guilliermondii</i>	<i>C. glabrata</i>
KOH	budding yeast and pseudohypha	budding yeast and pseudohypha	budding yeast and pseudohypha	budding yeast and pseudohypha
Gram's stain	gram positive	gram positive	gram positive	gram positive
Growth on SDA	creamy and white colonies	creamy and white colonies	creamy and white colonies	creamy and white colonies
GTT	+	-	-	-
CHROM agar	Light green and wet colonies	white color colonies	white color colonies	dark pink and wet colonies
Chamydospre formation	+	-	-	-
Zymogram test results				
Glucose	+	+	+	+
Lactose	-	-	-	-
Succrose	-	-	+	-
Trehalose	+/-	-	+/-	+
Maltose	+	-	-	-
Galactose	+/-	+/-	+	-

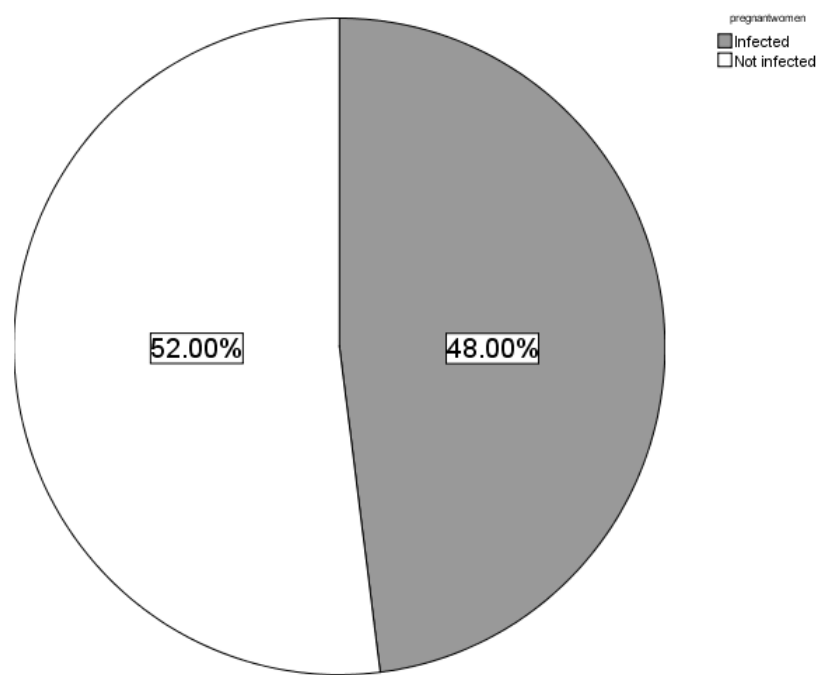


Figure 1. Frequency of vaginal candidiasis (VC) among pregnant women.

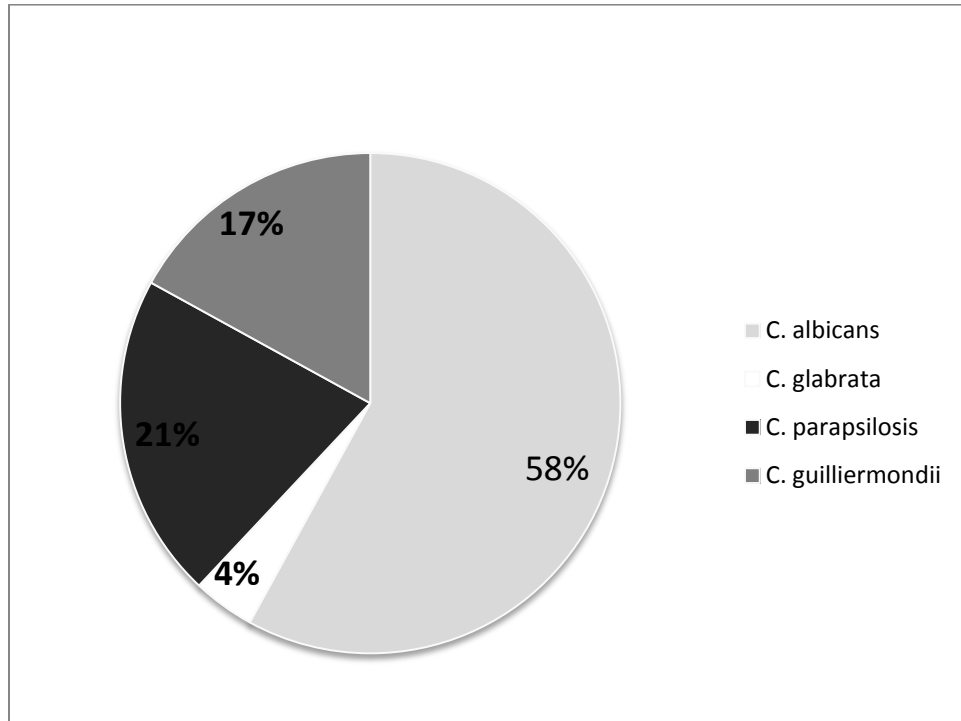


Figure 2. Frequency of different etiologies of VC among pregnant women.

Table 2. Vaginal Candidiasis and pregnancy trimesters among study population.

			Trimester			Total
			First trimester	Second trimester	Third trimester	
Pregnant women	Infected	Count	16	8	24	48
		% of Total	33%	17%	50%	48%
	Not infected	Count	2	14	36	52
		% of Total	4%	27%	69%	52%
Total		Count	18	22	60	100
		% of Total	18%	22%	60%	100%

P value= 0.001

*Sig value ≤ 0.05

Table 3. Vaginal Candidiasis and gravidity status among pregnant women.

			Gravidity		Total
			Primigravida	Multigravida	
Pregnant women	Infected	Count	16	32	48
		% of Total	33%	67%	48%
	Not infected	Count	24	28	52
		% of Total	46%	54%	52%
Total		Count	40	60	100
		% of Total	40%	60%	100%

P value= 0.191

*Sig value ≤ 0.05

Table 4. Vaginal Candidiasis and age ranges among pregnant women.

			Age group in years			Total
			18-25	26-33	34-41	
Pregnant women	Infected	Count	24	18	6	48
		% of Total	50%	37%	13%	48%
	Not infected	Count	16	26	10	52
		% of Total	31%	50%	19%	52%
Total		Count	40	44	16	100
		% of Total	40%	44%	16%	100 %

P value= 0.142

*Sig value ≤ 0.05

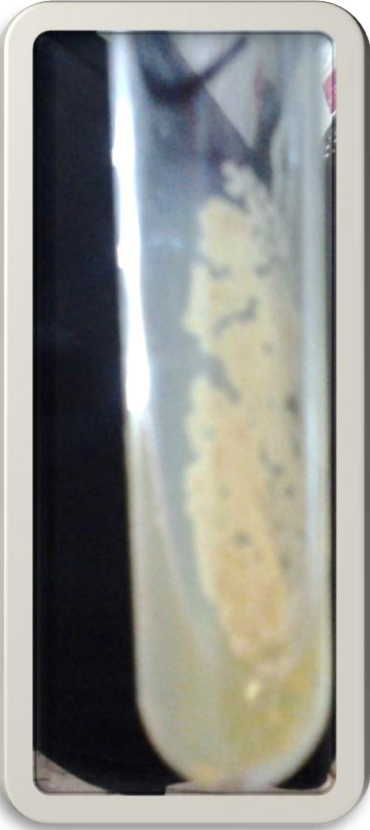
Table 5. Vaginal Candidiasis and taking antifungal treatment among pregnant women.

			Antifungal use		Total
			Yes	No	
Pregnant women	Infected	Count	12	36	48
		% of Total	25%	75%	48%
	Not infected	Count	4	48	52
		% of Total	8%	92%	52%
Total		Count	16	84	100
		% of Total	16%	84%	100%

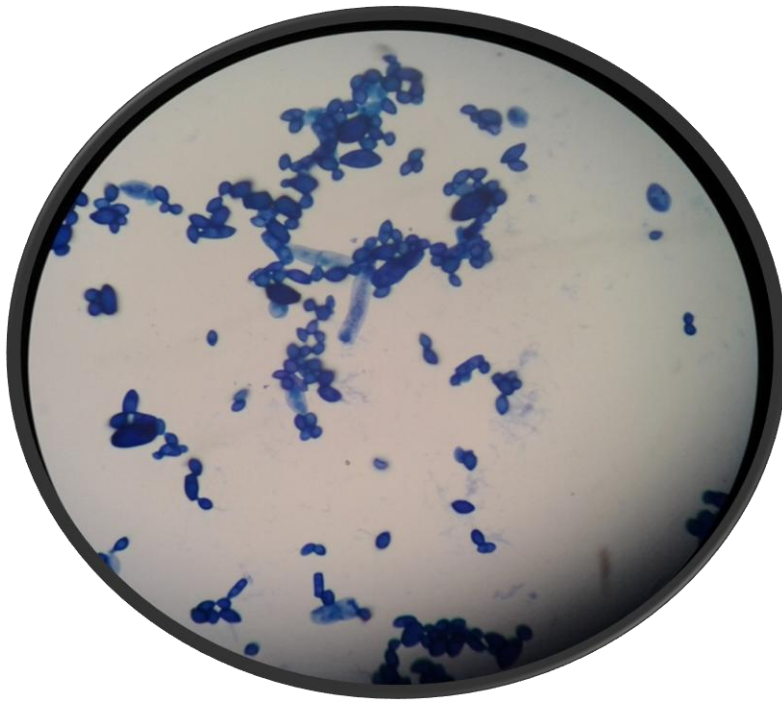
P value= 0.018

*Sig value ≤ 0.05

Candida grows as white, pasty colonies on SDA

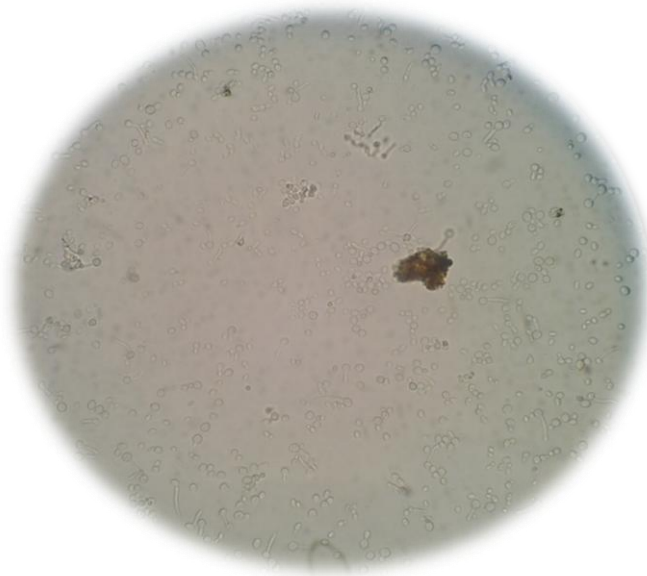


Gram's stain of *Candida*

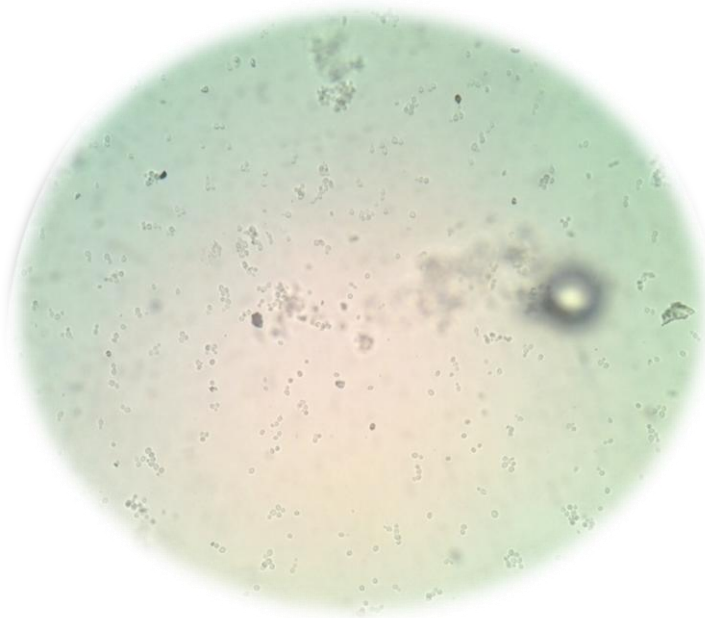


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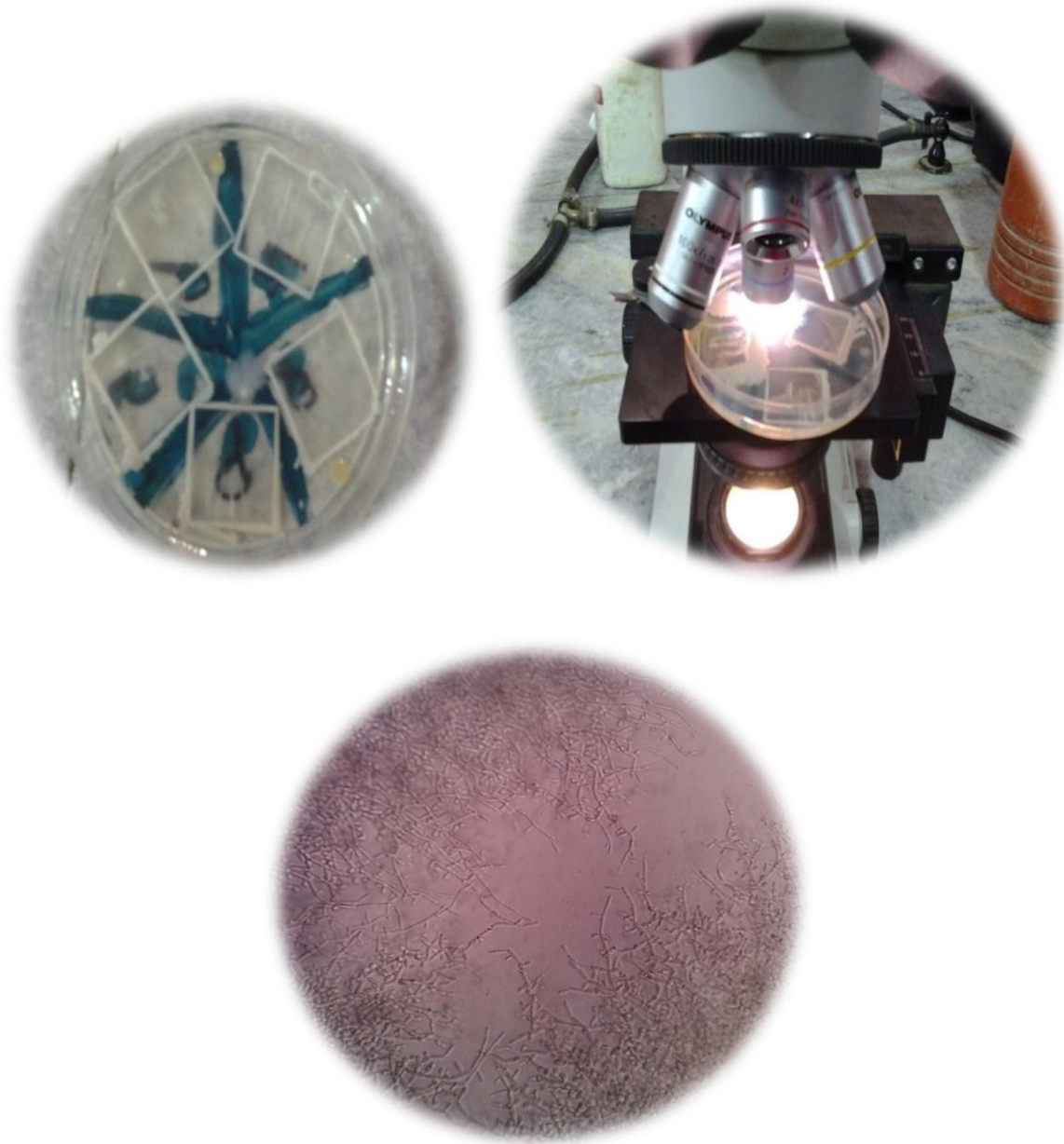
GTT of *Candida albicans* showed positive result



GTT of non -albicans spp showed negative result



Corn meal agar test for chlamydospore



Determination of *Candida* species on Chrom agar medium



CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

Vaginal candidiasis is a common complaint among women of different age groups in any society whether or not they are sexually active (Nwadioha *et al.*, 2010). Vulvovaginal Candidiasis in pregnant women is usually ignored in many countries (Aslam *et al.*, 2008).

The health of the mother during pregnancy is important to give birth of a healthy baby (Kamth, *et al.*, 2014).

In this study, the overall frequency of VC among pregnant women was 48%, *Candida albicans* predominates over other species (58%), *C. parapsilosis* is the second one most common (21%), but other species such as *C. guilliermondii* (17%) and *C. glabrata* (4%) are also encountered.

This is in agreement with other study conducted in Pakistan (2007), among pregnant women presenting in the antenatal clinic of a local private Hospital, Lahore during the last trimester of their pregnancy, mainly presenting with excessive vaginal discharge and itching (pruritis). *Candida* was isolated from 48% of all cultures. The common presenting signs and symptoms of VVC in their study were excessive vaginal discharge (100%), vaginal pruritis (91.6%) and vaginal burning (75%) (Aslam *et al.*, 2008).

Another study performed in Sarajevo, Bosnia (2006), 447 woman included in the study were separated in two groups: 203 pregnant (in the last trimester of pregnancy), and 244 non-pregnant woman in period of fertility. The results indicated positive microscopic findings in the test group (40.9%), as well as greater number of positive cultures (46.8%). the most commonly detected species for both groups was *C. albicans* (test group 40.9% and control group 23.0%). The most

commonly detected non-albicans species for the test group were *C. glabrata* (4.2%) and *C. krusei* (3.2 %), and for the control group were *C. glabrata* (3.2 %) and *C. parapsilosis* (3.2 %) (Babić and Hukić, 2010).

Our results regarding the predominant isolate is lower comparing with that of another study conducted in Kenya (2010), in which 104 samples obtained from the pregnant women attending the antenatal clinic of Thika District Hospital, their results indicated a high prevalence of vaginal candidiasis (42.7%). The percentage occurrence of vaginal *Candida* species showed that *Candida albicans* was the most isolated species with 60 (63.83%) isolates (Nelson *et al.*, 2013).

Other study showed that, vulvovaginal candidiasis is an inflammation of the vagina and vulva caused by *Candida albicans*, the prevalence of *Candida* spp. was 28% (*Candida albicans* 90.4%; *Candida glabrata* 6.3%; *Candida parapsilosis* 1.1%, *Candida kefyr* 1.1 %; unidentified species 1.1 %) (Garcia *et al.*, 2006).

In this study, the third trimester pregnant women are more infected (50%). The 1st and 2nd trimesters of pregnancy recorded low prevalent rates of vaginal candidiasis infection. The same finding had been documented by other investigators in India (2013), who found that (57%) of pregnant women who infected with VC in 3rd trimester, (10.65%) in 1st trimester and (32.4%) in 2nd trimester from a total of 142 pregnant women (Kamath *et al.*, 2014). Also other study conducted in Kenya (2010), found that 64 (68.09%) of infected pregnant women in the 3rd trimester of pregnancy. This may be explained the pregnant women in the 1st and 2nd trimesters could have less emotional stresses, high levels of vaginal defense mechanisms against *Candida* infections as a result of low levels of estrogen and corticoids hormones. Therefore, they have strong immune system against *Candida* species infections. These factors could have contributed to the low prevalence of the infection in these two trimesters of pregnancy (Nelson *et al.*, 2013).

In present study multigravida women are more infected with VC (67%) than primigravida (33%). Many results indicate that gravidity, as the risk factor for incidence of infection, has the significant role in the incidence of vaginal candidiasis (Babić and Hukić, 2010). This finding could be explained on the basis that multigravida has longer sexual history than the newly married primigravida and due to some alteration of the vagina, and so they are more prone to VVC than the later (Omar, 2001). The results are in agreement with a previous study conducted on 50 pregnant women in Pakistan (2007), multigravidae (60%) were more commonly affected than primigravidae (40%) (Aslam *et al.*, 2008).

Also another result had been documented by other investigators in Hyderabad (2005), who found that most of the women 91 (82.72%) were multigravida, and the rest of all 19 (17.27%) were primigravida. 84 (76.36%) women presented were in their third trimester of pregnancy and remaining 26 (23.63%) in second trimester (Parveen *et al.*, 2008).

In our study a higher frequency of vaginal candidiasis within different age ranges of the pregnant women was observed within the age ranges 18-25 years (50%) than the other age groups. A lower rate of prevalence of the infection in pregnant women was reported within the age ranges 34-41 years (13%).

Similar study was conducted on 110 pregnant women in Hyderabad (2005), and the results were analyzed according to their age, parity, trimester of pregnancy. Their result showed that 77 (70%) of pregnant women were between the ages of 18-30 and remaining 33 (30%) were between 31-40 years (Parveen *et al.*, 2008).

Another study conducted in Kenya (2010), showed that the percentage distribution of vaginal candidiasis within age group was highest in the age brackets 26 - 35 years with 56 (60%) patients (Nelson *et al.*, 2013),

Also in India (2013), from a total of 142 pregnant women, (39.4%) of infected pregnant women their age between 18 – 25 years, (52.8%) between 26 – 33 years and (7.7%) between 34 – 40 years (Kamath *et al.*, 2014).

In our study, (25%) of pregnant women who use antifungal treatment were infected with VC, and (29%) of pregnant women had history vaginal candidiasis.

The emergence of increasingly resistant *Candida* species does not seem to play a major part in recurrent vaginal candidiasis; women with recurrent disease do have higher prevalence (10–15%) of *Candida glabrata*, which is inherently less sensitive to the imidazole group of drugs (Janković *et al.*, 2010).

5.2. Conclusion

This study concluded that VC a disease of considerable importance during pregnancy as (48%) of pregnant women under study suffered from it.

Itching, pain and whitish vaginal discharge were the main signs and symptoms of VVC, but laboratory support is necessary for a differential diagnosis or to confirm the clinical diagnosis of vaginal candidiasis.

Candida albicans was the most prevalent vaginal *Candida* species across all age groups and trimesters.

Although there is generally a high frequency of vaginal candidiasis, an increased ratio of vaginal candidiasis in third trimester pregnant women requires these women to be routinely screened for vaginal candidiasis regardless of symptomatic status.

Antifungal therapy is one of the important tools to cure and help in eradication of vaginal candidiasis.

5.3. Recommendations

1. Diagnosis of infection should not be made on clinical criteria only, because some time women have another condition.
2. All patients have itching and vaginal discharge should be routinely diagnosed by culture.
3. Specimens must be collected by experienced health workers.
4. Treatment must be taken after full investigation and recommended by specialist.
5. Health education program should be introduced for all pregnant women for recommend prevention, early diagnosis and increase awareness about importance of treatment because multiple unreasonable miscarriage and neonatal death has been reported.

6. It is therefore recommended that further study be carried out to more research about VC especially recurrent infection and the validity of antifungal treatments that available and association with infertility, abortion and stillbirth.

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APPENDICES

Appendix [1]:

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

Frequency of Vulvovaginal Candidiasis among Pregnant Women with Whitish Vaginal Discharge Attending Bashaier Teaching Hospital

Demographic data: Name:.....Age:.....

- Trimester: First () Second () Third ()

- Gravidity: Primigravida () Multigravida ()

- Signs and symptoms: Discharge () Itch () Pain ()

-Medical history: First time () Recurrent ()

- Drugs and treatment consumption: Antibiotics () Antifungal ()

Laboratory investigation:

- Macroscopic examination: Color () Consistency () Odor ()

- Microscopic examination:

Wet preparation by 10% KOH.....Gram's stain.....

- Culture on SDA:.....

- Identification:.....

Appendix [2]: Potassium hydroxide, 200 g/l (20% w/v)

To make 50 ml:

Potassium hydroxide (KOH)	10 g
Distilled water	50 ml

Weigh the potassium hydroxide pellets. Transfer the chemical to a screw-cap bottle. Add the water, and mix until the chemical completely dissolved. Store it at room temperature. The reagent is stable for up to 2 years.

Appendix [3]: Gram stain reagents

Crystal violet

To make 1 litre:

Crystal violet	20 g
Ammonium oxalate	9 g
Ethanol or methanol, absolute	95 ml
Distilled water	1 liter

Weigh the crystal violet on a piece of clean paper (pre-weighed). Transfer to a brown bottle pre-marked to hold 1 liter. Add the absolute ethanol or methanol and mix until the dye is completely dissolved. Weigh the ammonium oxalate and dissolve in about 200 ml of distilled water. Add to the stain. Make up to the 1 litre mark with distilled water, and mix well. Store at room temperature.

Lugol's iodine solution

To make 1 litre:

Potassium iodide	20 g
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Iodine	10 g
Distilled water	1 liter

Weigh the potassium iodide, and transfer to a brown bottle premarked to hold 1 liter. Add about a quarter of the volume of water, and mix until the potassium iodide is completely dissolved. Weigh the iodine, and add to the potassium iodide solution. Mix until the iodine is dissolved. Make up to the 1 litre mark with distilled water, and mix well. Label the bottle, and store it in a dark place at room temperature.

Acetone-alcohol decolorizer

To make 1 liter:

Acetone	500 ml
Ethanol or methanol, absolute	475 ml
Distilled water	25 ml

Mix the distilled water with the absolute ethanol (ethyl alcohol) or methanol (methyl alcohol). Transfer the solution to a screw-cap bottle of 1 liter capacity. Measure the acetone, and add immediately to the alcohol solution. Mix well. Store in a safe place at room temperature. The reagent is stable indefinitely.

Safranin

For prepare safranin, 10g of safranin was weighed and transferred to a clean brown bottle. Then absolute ethanol (200ml) was added and mixed until the dye was dissolved, stored at room temperature.

To prepare the working solution, 100ml of safranin stock solution was taken to a clean bottle, then 400ml of distilled water was added and mixed.

Appendix [4]: Preparation of culture media:

Sabouraud's dextrose agar (SDA) (Hi Media Laboratories Pvt. Ltd. Mumbai, India)

Oxoid dehydrate medium formula (CN41)

Ingredients	Gms/ Litre
Mycological peptone	10 g
Dextrose	40g
Agar No.1	15 g

Final pH (at 25°C) 7.0 +/- 0.2

Preparation

The medium is used at concentration of 6.5 gram in every 100 ml of DW. Prepared and sterilize the medium as structured by manufacture. Allow to cool to 50-55°C, mix well and dispense aseptically in 15-20 ml amount in sterile petri dishes.

Chromogenic agar medium (Hi Media Laboratories Pvt. Ltd. Mumbai, India)

For differentiation between *Candida* spp. This medium is based on sabouraud dextrose agar (Oxoid CM41) and contain (per liter) 40.0 g of glucose, 10.0g of mycological peptone, and 15.0g of agar along with a novel chromogenic glucosaminidase substrate, ammonium 4-{2-[4-(2-acetamido-2-deoxy-β-o-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-(propan-3-yl-oate)-quinolinium bromide (0.32g/liter).

Corn Meal Agar medium (Hi Media Laboratories Pvt. Ltd. Mumbai, India)

Corn Meal infusion provides nitrogen, vitamins, minerals and amino acids essential for growth. Bacteriological agar is the solidifying agent.

Corn Meal infusion	50g
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Agar	15g
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Final pH 6.0 ± 0.2 at 25°C.

Preparation

Suspend 17 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121°C for 15 minutes. Cool to 50°C, mix well and dispense into Petri dishes.