



CANDIDIASIS AMONG PREGNANT WOMEN: A REVIEW

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Abstract

Candidiasis is a fungal disease caused by *Candida species* which is predominantly common in immune-compromised individuals and in pregnant women as well. *Candida species* are known causative agents of vaginal Candidiasis in pregnant women. Studies have shown that women in their 3rd trimester and multi-gravidae women are more commonly affected. Candida is the normal flora of human body but antibiotic over use, HIV/AIDS, diabetics, pregnancy, steroids and some cancer medications and contraceptive pills are predisposing factors to develop Candidiasis. Candidiasis has worldwide distribution. *Candida species* use several virulence factors that contribute to its pathogenesis such as adhesion, enzyme production, hyphal formation and biofilm formation. High prevalence of Candidiasis in pregnant women is associated with elevation of reproductive hormones which in turn impairs the immune response to pathogens including *Candida species*. Candidiasis has different clinical manifestations these are pruritis, thick crude/cheese like vaginal discharge, itching, redness, burning, swelling and pain during walking and urination. These clinical manifestations are the base for diagnosis of Candidiasis in pregnant women. In addition to clinical diagnosis, microscopy, culture and new molecular techniques like polymerase chain reaction (PCR) and rRNA gene clone library can be used for the diagnosis of Candidiasis. Seven days topical antifungal application is effective and safe for the treatment of Candidiasis in pregnant women.

Keywords: Candidiasis, Pregnant women.

Introduction

Candidiasis is a fungal disease caused by *Candida species* which is predominantly common in immune compromised individuals and also a known causative agent of vaginal Candidiasis in pregnant women^(1, 2). *Candida species* are the most common cause of fungal disease which range from simple mucocutaneous to life threatening invasive fungal infection⁽³⁾. The genus *Candida* is a taxonomic grouping that was originally used to define yeast-like organisms that were not

considered to have a sexual reproductive life cycle⁽⁴⁾. *Candida* contains over 350 heterogeneous species, but only minorities of these have been implicated in human disease⁽⁴⁾.

Among the different *Candida species* that cause vaginal Candidiasis in pregnant women, *Candida albican*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis*, *Candida dubliniensis*, *Candida pseudotropicalis*, *Candida*

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guillermoidii, and *Candida kyeare* commonly identified in pregnant women^(2,5,6,7).

Vaginal Candidiasis is common and frequent disease among women during their child bearing age; women with this age group face at least one episode of vaginal Candidiasis in their lifetime⁽⁸⁾. Number of gravida and stage of trimester have been associated with development of vaginal Candidiasis. Studies showed that women in their 3rd trimester and multigravidae women had the highest rate of Candidiasis occurrence^(2, 8).

Pregnant women are more susceptible to many diseases including Candidiasis due to immune suppression and hormonal imbalance; progesterone and estrogen are the known hormones that elevate during pregnancy. These two hormones suppress the normal functioning of the body's immune system⁽⁸⁾. *Candida* species are normally found in human body but there are factors that determine the organism to be pathogen to the host (human body). Virulence factors of the organism and/or predisposing factors of the host determine whether the organism remains as a commensal or become pathogen and causes disease^(8, 9). A number of predisposing factors to Candidiasis have been identified by different studies. Some of these factors include, HIV AIDS, steroids and some cancer medications⁽¹⁾, hot weather and tight clothing, obesity, pregnancy⁽⁸⁾, diabetes, birth control pills and overuse of antibiotics⁽⁹⁾.

Now a day Candidiasis among pregnant women is increasing due to several factors; so understanding of these factors will help the prevention, control and management of the disease among pregnant women. Therefore, the aim of this review was to document updates about the burden of the disease in pregnant women, the predominant *Candida* species involved, hormonal relations with pathogenesis and the virulence factor for the development of disease.

Virulence factors of Candida

Candida is common flora of human body in healthy individual however *Candida* expresses a variety of virulence factors that contribute to its pathogenesis for persistent infection and tissue damage of the host when immunity is debilitated^(10,11). Major virulence factors of *Candida* are its ability to adapt to a variety of

habitats of the body (oropharyngea, gastro intestine and female genitalia), adherence to host cells, the ability to switch between the yeast form and filamentous (pseudo hyphae formation), biofilm formation and production of hydrolytic enzymes such as proteinases, phospholipases, lipases and other factors play a major role in successful colonization and subsequent infection of *Candida*.^(4, 10, 11, 12, 13)

Ability to adapt different anatomical site

Candida species colonize and cause disease in different anatomical sites including skin, oral cavity and esophagus, gastrointestinal tract, vagina and vascular system by using different virulence factors. For example *Candida albicans* expresses PHR1 in blood stream or in tissue to adapt the neutral PH while it expresses RPH2 in the vagina to optimize and survive at acidic pH⁽¹²⁾.

Adherence of Candida to host surfaces (adhesion)

The ability of *Candida* to adhere to host surfaces is a prerequisite for both successful commensal carriage as well as persistence during active infection⁽¹¹⁾. *Candida* has the ability to adhere to several host cell types, including epithelial, endothelial and phagocytic cells. Among the different type of adhesions expressed by *Candida albicans*, agglutinin-like sequence which is consisted of several glycosylated proteins is very important for successful adhesion^(10, 11, 12).

Hyphal formation

The ability to switch between the yeast form and pseudo hyphal form is one of the virulence factor of *Candida* species⁽¹²⁾. Among the different species of *Candida*, *Candida albicans* and *Candida dubliniensis* are associated with the generation of hyphae⁽¹³⁾. Hyphae are believed to play an important role in tissue and biomaterial invasion. Study reported that, species that do form hyphae have high ability to invade tissue and are resistant to phagocytosis⁽⁴⁾. Hence, hyphal formation is considered to be significant to the pathogenicity of *Candida*.

Biofilm formation

Biofilms can be defined as microbial communities or aggregation of microorganisms that are often (but not necessarily) attached to a solid surface. *Candida* strains that have the ability to form biofilms are more virulent than others; this has

been associated with increased expression of virulence factors as well as reduced susceptibility to antimicrobial agents⁽⁴⁾.

Production of hydrolytic enzymes

Candida produces several hydrolytic enzymes including secreted aspartyl proteinases, phospholipases, lipases, phosphomonoesterase and hexosaminidase. These enzymes have the capacity to degrade human proteins and help the organism to invade the human body easily hence are associated with tissue invasion^(4,11,12).

Pathogenesis

The genus *Candida* has diverse species that are common residents of soil and mucosal surfaces of human gastrointestinal tract, genito-urinary tract and the mouth⁽¹⁴⁾. But there are a number of factors that favors the organism to be pathogen to the host; the factors may be virulence factor of the organism or predisposing factor of the host⁽⁹⁾. The common predisposing factors of the host are: broad spectrum antibiotic treatment, chronic corticosteroid therapy, diabetes, pregnancy, organ transplantation, Human Immunodeficiency Virus⁽¹⁵⁾, oral contraceptive pills and contraception devices (diaphragm, vaginal contraceptive sponge, intrauterine device)⁽¹⁶⁾. All these factors have their own impact on host either by directly suppressing the host immunity, inhibit the normal flora of the body that competes pathogen or by disturbing the normal physiology of the human body which results in progress of disease condition^(15,16).

During pregnancy the level of estrogen and progesterone increase. This results in immune suppression for example; progesterone has suppressive effect on neutrophils⁽⁸⁾. As studies indicated, neutrophils have important role to defend the host from *Candida* infection by oxidative and non-oxidative killing mechanisms and also neutrophils are the only effector cells that can inhibit the development of *Candida* from yeast-to-hyphae using oxidative burst mechanisms this is very crucial to prevent invasive *Candida* formation⁽¹⁰⁾. But due to high production of progesterone during pregnancy, the normal functioning of neutrophils is impaired which in turn increases *Candida* colonization and infection. In addition high production of estrogen during pregnancy has its own impact on immunoglobulin secretion in the vagina which results in mucosal

surface defense failure due to lack of immunoglobulins⁽⁸⁾.

Generally, the main reason for increased vaginal Candidiasis in pregnant women is due to elevation of reproductive hormones during pregnancy. Study revealed that vaginal Candidiasis is very rare in post-menopausal women and girls⁽¹⁷⁾. But high levels of reproductive hormones during pregnancy provide an increased amount of glycogen in the vagina which enhances growth of *Candida species* because glycogen serves as a carbon source for *Candida* growth^(17, 18). In addition estrogen enhances adherence of yeast to vagina by reducing immunoglobulin secretion and fungal inhibition activity of epithelial cells⁽⁸⁾.

Clinical manifestation

Candidiasis shows different signs and symptoms depending on the site of infection. For example *Candida* infection in the skin will manifest patches on skin, itchy of skin, scabs and pustules may be seen around the edge of the rash. These clinical manifestations are commonly seen around groin, the folds of the buttocks, between the breasts, toes, or fingers. Candidiasis in oral cavity causes curd-like white patches inside the mouth, on the tongue and palate and around the lips. Clinical manifestations of Candidiasis in pregnant women are vaginal pruritis, thick crud/cheese like vaginal discharge, itching, redness, burning, swelling and pain during walking and urination^(8, 9, 19). Candidiasis clinical presentation based on anatomical site of infection is presented here below:

Oropharyngeal Candidiasis

Oropharyngeal Candidiasis commonly called oral Candidiasis or oral thrush is an opportunistic yeast infection of the mouth, pharynx, tongue, and buccal mucosa. Oral Candidiasis is the predominant opportunistic infection in HIV infected patients and *Candida albicans* is the most common species identified from Oropharyngeal Candidiasis⁽²⁰⁾. Oropharyngeal Candidiasis can present in one of four forms: Pseudomembranous Candidiasis, Erythematous Candidiasis, Hyperplastic Candidiasis, and Angular cheilitis are commonly observed clinical manifestation of oral Candidiasis⁽¹¹⁾.

Candidemia

Candidemia or systemic Candidiasis is the infection of blood by *Candida species*. Invasive Candidiasis or candidemia is the most frequent cause of morbidity and mortality in hospitalized patients⁽¹⁵⁾. Invasive Candidiasis is increasing in the past two decades associates with medical advances such as invasive medical devices, organ transplantation, intravascular catheters, intensive cancer chemotherapy, broad spectrum antimicrobial therapy; mechanical ventilation and prolonged hospital stay are the common risk factors in addition to HIV/AIDS. Study reported isolation of *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, and *Candida Krusei* from patients with systemic Candidiasis. Invasive Candidiasis is the fourth leading cause of nosocomial bloodstream infection in the United States^(10,15).

Vaginal Candidiasis

Vaginal Candidiasis is the most common type of Candidiasis in pregnant women .The clinical manifestation of the disease is characterized by vaginal pruritis, thick crud/cheese like vaginal discharge, itching, redness, burning, swelling and pain during walking and urination^(8, 9). These clinical signs and symptoms, though not specific, are important for the diagnosis of vaginal Candidiasis. Pregnant women with such clinical manifestations should be treated as early as possible because Candidiasis is reported as a risk factor for preterm birth⁽²¹⁾. Vaginal Candidiasis can be classified as either uncomplicated or complicated. According to Center for Disease prevention and Control (CDC), approximately 10%–20% of women with vaginal Candidiasis will have complicated type that necessitates diagnostic and therapeutic considerations. Uncomplicated type of Candidiasis is sporadic or infrequent, mild-to-moderate vulvo vaginal Candidiasis and mostly

caused by *Candida albican* and this may occur in immuno competent women while complicated form of vaginal Candidiasis is severe form and recurrent infection is common. In complicated form of Candidiasis, non albican *Candida species* are common and the disease is commonly seen in diabetic, debilitated or immunosuppressed women. The outcome of complicated vaginal Candidiasis is psychosexual problems (e.g. feeling unclean or not wanting to have sex) and depression⁽²²⁾.

Epidemiology

Vaginal Candidiasis is the most common disease in pregnant women all over the world. The risk of carriage to develop disease in pregnant women is two times higher than non-pregnant women particularly during the third trimester due to changes in the levels of reproductive hormones and deposition of glycogen in the vagina^(23,24). Studies show that Candidiasis is the most common cause of vaginitis; in United States it is the second leading cause of vaginitis next to bacterial vaginosis and it is also common in Europe⁽²³⁾. Study from Spain reported 28% prevalence of *Candida species* among pregnant women. Of these, *Candida albicans* was the major isolate 90.4% followed by *Candida glabrata* 6.3%; *Candida parapsilosis* 1.1% and *Candida kefyr* 1.1 %⁽⁷⁾. Other studies around the world also reveled high prevalence of the disease; Sarajevo 46.8%⁽¹⁷⁾, Cuba 42.3%⁽²³⁾ and Kenya 42.7%⁽²⁾. Most studies reported *Candida albican* as the most predominant cause of vaginal Candidiasis in pregnant women which accounts 80-95% of *Candida* infection^(2, 5, 11, 17,18). From non albican group, *Candida glabrata* is the leading cause of vaginal Candidiasis in pregnant women⁽¹⁷⁾.

In Ethiopia however, epidemiological studies of Candidiasis are limited to HIV patients and diabetic patients. Hence, literatures about the epidemiology of Candidiasis among pregnant women are lacking.

Table No. 01: Prevalence of Candidiasis in pregnant women among different countries around the world

S. No	Country	Year of study (Publication)	Total no of study population	Total no of people with Candidiasis No (%)	References
1	Nigeria	2013	60	42(70%)	(24)
2	Nigeria	2010	8443	2458 (29.1%)	(25)
3	Saudi Arabia	2012	495	120(24.24)	(26)
4	Pakistan	2008	50	24(48%)	(8)
5	India	2013	250	121(48.4%)	(27)
6	Mexico	2012	167	35 (20.9)	(28)
7	Nepal	2011	200	78 (29.5%)	(29)

During this review, there were few studies on Candidiasis in Jimma⁽³⁰⁾, Addis Ababa⁽³¹⁾ and

Gondar⁽³²⁾ among HIV patients and among diabetic patient in Gondar⁽³³⁾ as well.

Table No. 02. Common *Candida* species identified from pregnant women

Sr. No	<i>Candida</i> Species	Year	Country	%	Reference
1	<i>Candida albicans</i>	2012	Kenya	63.8%	(2)
		2010	Bosnia and Herzegovina	40.9%	(17)
		2006	Spain	90.4%	(7)
2	<i>Candida glabrata</i>	2012	Kenya	29.79%	(2)
		2010	Bosnia and Herzegovina	4.2%	(17)
		2006	Spain	6.3%	(7)
3	<i>Candida tropicalis</i>	2012	Kenya	3.19%	(2)
4	<i>Candida krusei</i>	2012	Kenya	2.13%	(2)
5	<i>Candida dubliniensis</i>	2010	Bosnia and Herzegovina	3.2%	(17)
		2007	Turkey	2.43%	(6)
6	<i>Candida kefyr</i>	2006	Spain	1.1%	(7)
7	<i>Candida parapsilosis</i>	2012	Kenya	1.06%	(2)
		2006	Spain	1.1%	(7)

Diagnosis of Candidiasis

Candidiasis can be diagnosed based on the clinical feature of the disease. Although the signs and symptoms of Candidiasis are relatively non-specific, clinical manifestations of *Candida* infection serve as a diagnostic approach of the disease^(34, 35,36). Microscopic examination of normal saline or 10% potassium hydroxide wet mount preparation and gram stained preparation from vaginal discharge of clinically positive pregnant women are basic tests for the diagnosis of Candidiasis^(8, 18, 36). Microscopic examination identifies only the presence of *Candida* but do not isolate the species. Hence, further identification methods are necessary to identify the species of *Candida* such as culture using Sabourand dextrose agar (SDA) which can support growth of *Candida* and suppresses the growth of many bacteria due to its low pH and antibiotic content^(2,6). It is also possible to use CHROM agar to detect mixed cultures⁽¹⁷⁾. CHROM agar is a fungal culture media which can be used to identify species based on the reaction between different species of *Candida* and chromogenic substrate after incubation at 37 °C; different *Candida* species produce different type of colonies with different color⁽¹⁷⁾. Germ tube test is also another type of *Candida* species identification method which helps to identify hyphae former *Candida* species such as *Candida albican* and *Candida dubliniensis*; hyphae formers produce hyphae when incubated at 35 °C in serum for 2-4 hour^(6, 17). Moreover, yeast assimilation test is the safest identification method of non albicans species. This test is based on the ability of yeast to assimilate organic compounds⁽¹⁷⁾.

Diagnosis of invasive Candidiasis is not simple because clinical presentation of invasive Candidiasis is not specific and up to 50% blood cultures can be negative or may only become positive late in the infection. Hence invasive Candidiasis can be diagnosed using different methods such as antibody detection, though it is not sensitive in immune compromised individuals and nonspecific to superficial colonization. Detection of antigens such as extracellularly secreted aspartyl proteinase which is produced by *Candida albicans* and some other *Candida* species are target for diagnosis of invasive Candidiasis. Histo pathological examination of tissue sections is also one of the most reliable methods of establishing a diagnosis of systemic fungal infection⁽³⁷⁾.

Rapid test kits are also available for the diagnosis of *Candida*; for example Immunologic Latex Agglutination Test is the one used for *Candida* diagnosis⁽²³⁾. Now days there are also more sensitive and specific molecular techniques such as polymerase chain reaction (PCR) and 18S rRNA gene clone library methods which allow identification of drug resistant *Candida* in addition to species identification. The basic principle of PCR is amplifying of the target DNA in high amount with in different temperature ranges^(28, 33,38).

Vulvovaginal Candidiasis diagnosis and treatment algorithm indicated physical examination for abnormal pelvic findings as a primary step. When the patient has abnormal pelvic finding direct microscopy will be considered. Those positive for

direct microscopy plus pH value of discharge <4.5 and no excess of white blood cells in the discharge, antimycotic treatment will be initiated. On the other hand in the presence of positive microscopic examination, if pH of discharge is >4.5 and excess of white blood cells exist in the discharge, mixed infection is considered. When the patient is negative for microscopic examination and the pH of the discharge is <4.5 with no excess of white blood cells in the absence of trichomonads infection and clue cells, while culture considered antimycotic treatment will be initiated for the patient⁽¹⁸⁾.

Despite the availability of different diagnostic modalities, in Ethiopia however, Candidiasis is being diagnosed clinically and through microscopic identification of the yeast in clinical samples. Therefore, evaluation of different diagnostic modalities and selection of the best Candidiasis diagnostic method among pregnant women in developing countries like Ethiopia has a paramount significance for proper management of the disease.

Treatment of Candidiasis in pregnant women

Antifungal drug choice is determined by several factors including the patient's medical history, specific symptoms of Candidiasis, severity of infection, predicted compliance with application method, and drug sensitivity pattern of the isolated *Candida species*⁽³⁵⁾. There are several types of antifungal agents commercially available for the treatment of Candidiasis which include the following: imidazole antifungals (eg, butoconazole, clotrimazole, miconazole), triazole antifungals (eg, fluconazole, terconazole), and polyene antifungals (eg, nystatin). Study conducted in 1997 recommend topical antifungal agents for the treatment of Candidiasis in pregnant women while it discourages use of oral antifungal agents because of fetal complications⁽³⁵⁾. To the contrary, prospective and observational studies proves that using these antifungal drugs during pregnancy is not associated with increased risk of major malformations although there was case report due to high dose of oral antifungal agents⁽³⁹⁾.

Other study reported that even though it is possible to use many type of topical antifungal agents, topical imidazole appears to be more effective than nystatin for treating symptomatic vaginal Candidiasis in pregnancy⁽⁴⁰⁾. Different studies

agree on seven days antifungal topical therapy as the best for treating Candidiasis in pregnant women rather than shorter duration since short term therapy is associated with treatment failures^(39,40). Treating of *Candida* in pregnant women has significant role to reduce preterm birth in addition to maintaining mothers' health. Study showed that *Candida* colonization is a risk factor for preterm birth and infant mortality; hence treating of pregnant women reduces preterm birth^(2, 41). Retrospective analysis of data in Hungary revealed that 34-64% of preterm birth was prevented by applying vaginal clotrimazole treatment of pregnant women who had vaginal Candidiasis⁽⁴²⁾.

Antifungal susceptibility testing can be performed using different methods such as broth microdilution method this can be performed as follows : prepare inoculums suspension adjusted to a 0.5 McFarland Standard, dilute the working suspension into 1:100 dilution, add 10 μ l suspension with 10 μ l sterile normal saline then mix suspension and pour into an inoculation tray in Tryptose Soya Broth, then shake the microtiter plate on a plate shaker for 30 seconds to ensure even distribution of the inoculums, incubate at 37°C for 48 hours, take out the microtiter plate from the incubator and shake for 5 minutes. Finally the minimum inhibitory concentrations will be determined by reading the optical density of each well at 530 nm spectrophotometrically^(43, 44). The second method is using modified disc diffusion method, by adding 2% glucose and methylene blue to Mueller-Hinton agar to accelerate growth of *Candida* and inhibit the growth of bacteria. The test can be performed as follows: prepare colony from an overnight subculture on SDA suspension in sterile normal saline and vortex (mix) which should be comparable to 0.5 McFarland Standard, then dilute 1:2 with sterile saline solution, then inoculate the Mueller-Hinton agar with moistened cotton swab then allow to dry for around 15 mint, place antifungal discs on surface of Mueller-Hinton agar then incubate the plates at 37°C for 24 hours finally measure the zone of inhibition using ruler⁽⁴⁵⁾.

Empiric antifungal therapy to treat vaginal Candidiasis could act as risk factor for increased resistance of *Candida species* to antifungal agents; since all *Candida* species do not have equal susceptibility pattern. For example antifungal susceptibility studies done in Kenya and Brazil

revealed that *Candida krusei* was resistant to ketoconazole^(2,44). Hence during treatment of Candidiasis antifungal susceptibility test is necessary because all *Candida species* may not be susceptible to different antifungal drugs. Since drug resistance pattern of *Candida species* is variable to the different types of drugs used for the treatment of Candidiasis, susceptibility testing is very important to select appropriate antifungal agents. Study conducted to determine antifungal susceptibility of different *Candida species* revealed that *Candida albicans*, *Candida kefyr* and *Candida parapsilosis* were susceptible to fluconazole, ketoconazole, itraconazole and nystatin while 1 in 6 *Candida glabrata* isolates showed resistance to azole drugs⁽⁷⁾. Other study conducted to determine the susceptibility pattern of *Candida species* result reported that *Candida albicans* was susceptible to Clotrimazole while resistance to Fluconazole, Ketoconazole, and Econazole was detected. Most of other *Candida species* show resistance against azoles specially among most isolates of *Candida glabrata* was resistant to Ketoconazole, Clotrimazole, Fluconazole and Econazole^(44,45).

Conclusion

Pregnant women are more vulnerable to vaginal Candidiasis due to reproductive hormone elevation which suppresses the normal activity of the body's immune function and enhances the growth of *Candida* by increasing glycogen level in the vagina. Although Candidiasis has different clinical presentations vaginal Candidiasis is the predominant one in pregnant women. Candidiasis can be diagnosed using a combination of different methods such as clinical sign and symptom, microscope, culture, serology (antigen & antibody detection), germ tube test, sugar assimilation and PCR. Antifungal susceptibility testing can be performed using broth micro-dilution or modified disc diffusion Mueller-Hinton agar method. Empirical antifungal therapy is a risk for drug resistance among *Candida species*. Most of non *albicans Candida species* are resistant to azole drugs. Seven days treatment is necessary to cure pregnant women and effective treatment reduces the risk of preterm birth.

Recommendations

Vaginal Candidiasis is common in pregnant women therefore early diagnosis and treatment should be practiced. Antifungal treatment should be based on

drug sensitivity pattern of *Candida species* isolated because some *Candida species* are resistant to one or more antifungal agents. During this review, there were no literatures that show epidemiology of Candidiasis among pregnant women in Ethiopia. Therefore, epidemiological studies regarding Candidiasis among pregnant women have paramount significance.

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