Full Length Research Paper

Prevalence of Vulvovaginal Candidiasis in Pregnancy

*Khushbu Yadav[1] and Satyam Prakash[2]

[1*]Medical Microbiologist & Lecturer, Krishna Medical Technical Research Center, Purwanchal University, Janakpurdham, Nepal
[2]Assistant Professor, Department of Biochemistry, Janaki Medical College Teaching Hospital, Tribhuvan University, Janakpurdham, Nepal

Transmission of *Candida* infections can occur from the vagina of the infected mother to the newborn, giving rise to congenital *Candida* infection. Vulvovaginal candidiasis (VVC) is an important cause of morbidity in pregnant women. It has been related to emotional stress and suppression of immune system which steps up the risk of *Candida* species overgrowth and become pathogenic which can cause abortion, *Candida* chorioamnionitis and subsequent preterm delivery. Therefore, the present study was focused to determine the contributory agent of VVC and to determine its prevalence among pregnant women. A cross-sectional descriptive study comprised of 157 pregnant women of reproductive age group was selected. Two high vaginal sterile cotton swabs were collected from the pregnant women and were immediately brought to the laboratory. Culture media were prepared as instructed by the manufacturer company (Hi-media) and processed according to standard methods. The prevalence of vulvovaginal candidiasis was found to be 35%. The highest number of VVC was found within the age group of 21-25 years with 40.44%. *C. albicans* was found to be predominant organism to cause candidiasis. Most of the respondents had suffered from candidiasis in 2nd trimester of gestation period of 55% and those who had symptoms of VVC. Most of the respondents had illiterate and unemployed of high positive cases with 35.95% and 44.94% which was found to be statistically significant (p= 0.001) and insignificant (p= 0.328) respectively. The prevalence of vulvovaginal candidiasis in the pregnant women was in increasing trend. Therefore, routine medical examination, adequate ante-natal services and appropriate treatment of the infected women should be recommended which can prevent the complications associated with VVC.

Key words: Vulvovaginal candidiasis, Pregnancy, *C. albicans,* Gestation, Significant

INTRODUCTION

Women are considered as a supreme creature of God undergoes through a variety of physiological changes during pregnancy (Prakash and Yadav, 2015). Pregnancy is a dynamic state that normal fetal development needs the availability of essential nutrients such as glucose, free fatty acids, long-chain polyunsaturated fatty acids, amino acids, minerals and vitamins are to be continuously supplied to the growing fetus despite intermittent maternal food intake (Butte, 2000; Prakash *et al.,* 2015). The female genital tract (FGT) is considered as the point of entry for number of pathogens for various sexually and non-sexually transmitted diseases that affect the FGT causing vaginal discharge. Vaginal discharge is a common symptom in gynecological clinic and is often

the second most common gynecological problem after menstrual disorders (Akinbami *et al.,* 2015).

Vulvovaginal candidiasis (VVC) is also called candidal vaginitis or monilial infection of the vulva which is caused by overgrowth of candida yeast species, most commonly *Candida albicans*, *C. glabrata* and *C. tropicalis* characterized by curd like vaginal discharge, itching, erythema, burning, irritation of vulva and vagina usually odourless with dysuria and dyspareunia (Akinbami *et al.,* 2015; Rathod *et al.,* 2015; Nviriesy, 2008). *Candida* species that rarely cause infection include *C. parapsilosis, C. pseudotropicalis, C. krusei, C. guilliermondi* and *C. stellatoidea* (Cronje *et al.,* 1994; Mitchell, 2004). Candida species are almost universal in low numbers on healthy adult skin and are part of the normal flora of the mucous membrane of the respiratory, gastrointestinal and female genital tracts (20-50%). Overgrowth of these organisms can cause superficial infections such as vaginitis, most common form of typically associated with an immuno-compromised state mucosal candidiasis (Azaz, 2005).

Pregnant women are more vulnerable to VVC than healthy women with chronic recurrent candidiasis (Mitchell, 2004). The infection can be acute, chronic, superficial or deep and has broad clinical spectrum. The increased estrogen level during pregnancy leads to the production of more glycogen in the vagina which allows for the proliferation of yeast cells on the wall of the vagina (Parveen, 2008). However, any physiological changes that affect the beneficial bacteria in the vagina would alter the acidity of the vagina reducing its p^H to 5.0-6.5 by enhancing the establishment of pathogenic organisms such as *Candida* (Akinbiyi, 2008). Vaginal p^H may increase with age, luteal phase of menstrual cycle, sexual activity, oral contraception choice, pregnancy and use of antibiotics (Ohmit *et al.,* 2003; Leon *et al.,* 2002; Gonzalez *et al.,* 2008).

It has been estimated that up to 40% of pregnant women worldwide may have VVC (Alo *et al.,* 2012; Alli *et al.,* 2011). 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 during their life time (Azaz *et al.,* 2005; Sobel, 2003; Aslam, 2008; Fidel and Cutright, 2000). Several risk factors can be associated with the increased role of colonization of vagina by Candida species in women which includes compromised immune system, obesity, diabetes, prolonged use of broad spectrum of corticosteroids, HIV/AIDS, pregnancy, poor dietary habits, use of high level of estrogens and Oral Contraceptives Pills (OCPs), Intrauterine Contraceptive Device (IUCDs), tight clothing, use of vaginal douches, poor personal hygiene, use of sponge, intrauterine devices, diaphragms, condoms, sexual intercourse and diet with high glucose content (Akah *et al .,* 2010; Reed *et al.,* 2003). About 5-10% healthy women apparently suffer from recurrent vaginal candidiasis without any predisposing factors (Mitchell, 2004).

Vulvovaginal candidiasis is an important cause of morbidity in pregnancy which can cause abortion, candida chorioamnionitis, subsequent preterm delivery, emotional stress and suppression of immune system (Sobel, 1997; Sobel, 1985; Singh, 2003). Vaginal symptoms are one of the most common reasons for gynecological consultation accounting for approximately 10 million office visits each year (Feyi-Waboso and Amadi, 2001). Many women think their symptoms are normal occurrence or are reluctant (Reed *et al.,* 2003). Although, the problem may seems small but for the sufferer it is the immense physical and psychological problem that may require instant attention, which is lacking in the most of the hospitals and clinics.

In Nepal, very few studies have been implicated in the prevalence and therapeutic consideration of VVC. So, it is troublesome to know how frequent this disease is common among Nepalese pregnant women due to the dearth of information. It has been reported that one in three women wants consultation for vaginal discharge (Sobel, 1985). But nowadays, the number of serious opportunistic yeast infections, particularly in immuno-compromised patients, has dramatically increased (Richardson and Warnock, 2003). Therefore, the present study was focused to determine the prime importance of causative agent of VVC and prevalence of this disease among Nepalese pregnant women which may be helpful to disseminate knowledge regarding VVC as many are unaware of it and for the prevention from re-infection, stillbirth, abortion and sterility to the women.

MATERIALS AND METHODS

Study Design

A cross-sectional descriptive study was done among the pregnant women in Department of Microbiology, Clinical Pathology Laboratory, Janaki Medical College Teaching Hospital, (JMCTH), Janakpur, Nepal from August 2014 to January 2015. This study comprised of 157 women of reproductive age group who were pregnant.

Ethical Consideration

Informed verbal consent was obtained from the participants prior to the study before proceeding the questionnaire and specimen collection and work approval was taken from the institutional ethical committee of JMCTH.

Acceptance Criteria of Respondents

Pregnant women on attending antenatal at the hospital were included while members of staff of the hospital, patients with previous history of preterm labor or spontaneous abortion and those that did not give their consent were excluded from the study.

Specimen Collection and Transportation

Gynecologist, trained nurse and matron helped in collecting two high vaginal sterile cotton swabs from the posterior fornix of the pregnant women. One swab was inserted in the labeled sterile capped test tube and capped tightly for whiff test. And another swab was inserted in the labeled sterile test tube containing 0.5 ml of normal saline for the culture, wet mount and gram staining. The collected samples were immediately brought to the laboratory and processed according to standard methods.

Specimen Processing

Of two collected vaginal swab, one swab was used for the preparation of whiff test and another swab was used for culture, wet mount and gram stain. For Whiff test, the swab was rolled on the clean, dry, grease free new slide and a drop of 10% KOH was added to it. The slide was hold close to the nose to detect the amine odor. If fishy smell noticed then the sample was considered as positive. The specimen would quickly become odorless upon standing so the odor should be noted as soon as possible.

Culture media were prepared as instructed by the manufacturer company (Hi-media). A loopful of inoculum from normal saline was streaked on Sabaraud Dextrose Agar (SDA) plate. Plate was then incubated at 37ºC for 24 – 48 hours. The significant growth was observed and colony morphology was noted. Grams staining were performed. If the yeast cells were seen in staining then it was proceed for the germ tube test.

Identification of Candida species

Isolates from Saboraud dextrose agar (SDA) were inoculated on CHROMagar (Oxoid, Basingstoke, UK) using an inoculating loop and incubated at 37°C for 48 hours. Identification of yeast was done based on the colour of each colony. Using this method, the following *Candida* species were identified; *C. glabrata* (wet dark pink colonies), *C. tropicalis* and *C. dublinensis* (wet blue colonies) and *C. albicans* (wet green colonies).

Germ tube test

For Germ tube test, 500 µl of human serum was pipette into small test tube. Using a sterile wire loop, the serum with a yeast colony from the SDA plate was inoculated. The tube was incubated at 35 - 37º C for 2-3 hours. Using the Pasteur pipette, a drop of serum yeast culture was transferred to a glass slide and covered with a cover glass. Tube-like outgrowths from the cells was examined using the 10X and 40X objectives (Figure 1).
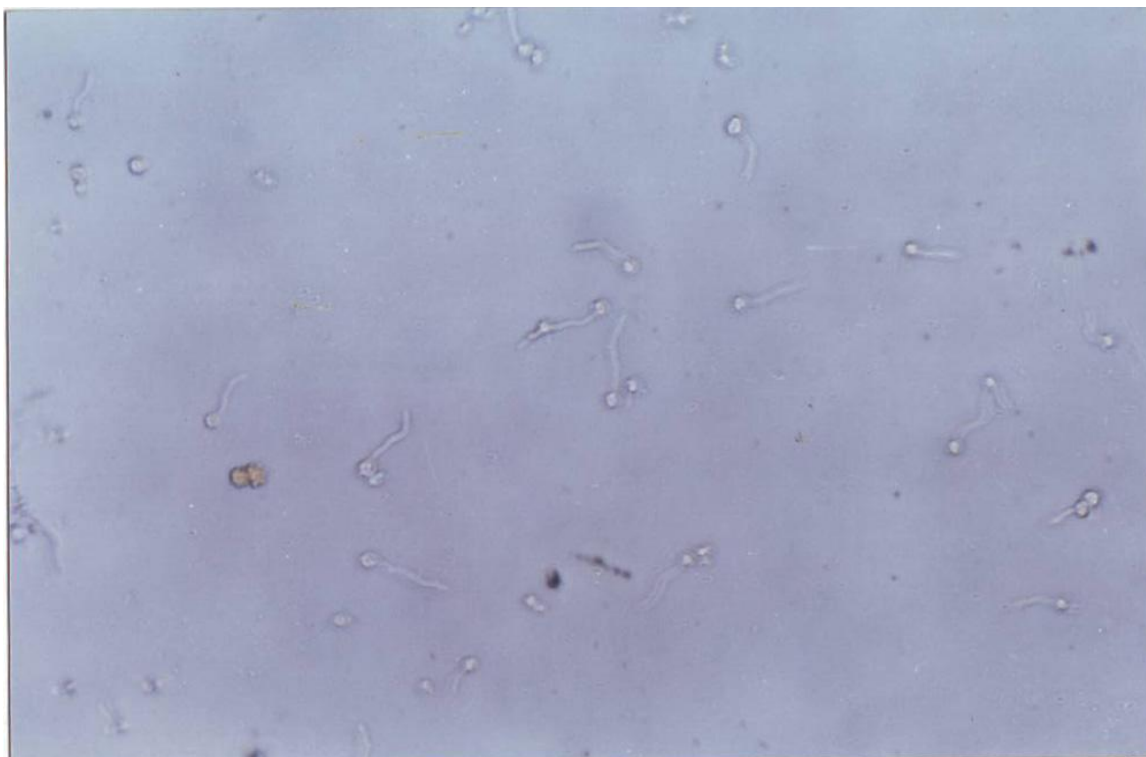

Figure 1: Germ tube test of *C. albicans* (D56) (40X)

For wet mount, a drop of normal saline was added to the center of the clean, dry, sterile, grease free slide and covered with cover slip. Clue cells and yeast cells were observed in microscopy.

Safe disposal of sample and contaminated tools

After handling of the specimen, it was discarded in the specific container and all the equipments used was placed in the beaker containing lysol, cleaned and then was sterilized. The swabs were incinerated.

Statistical Analysis

Opened self prepared questionnaire was administered to collect the data from pregnant women. The data were analyzed using statistical package for SPSS 17.0 version and Microsoft excels 2007. The p-value < 0.05 was considered as statistically significant.

RESULT

Prevalence of Candidiasis among total study population

A total number of 157 pregnant women were suspected as vulvovaginal candidiasis by Gynecologist. Of total, the prevalence of vulvovaginal candidiasis was found to be 35%. The results are shown in Figure 2.

Age wise distribution of pregnant women with Positive cases

The highest numbers of positive cases were found to be in 21-25 years age group of 40.44% followed by 26-30 years with 32.58%. The results are shown in Table 1.
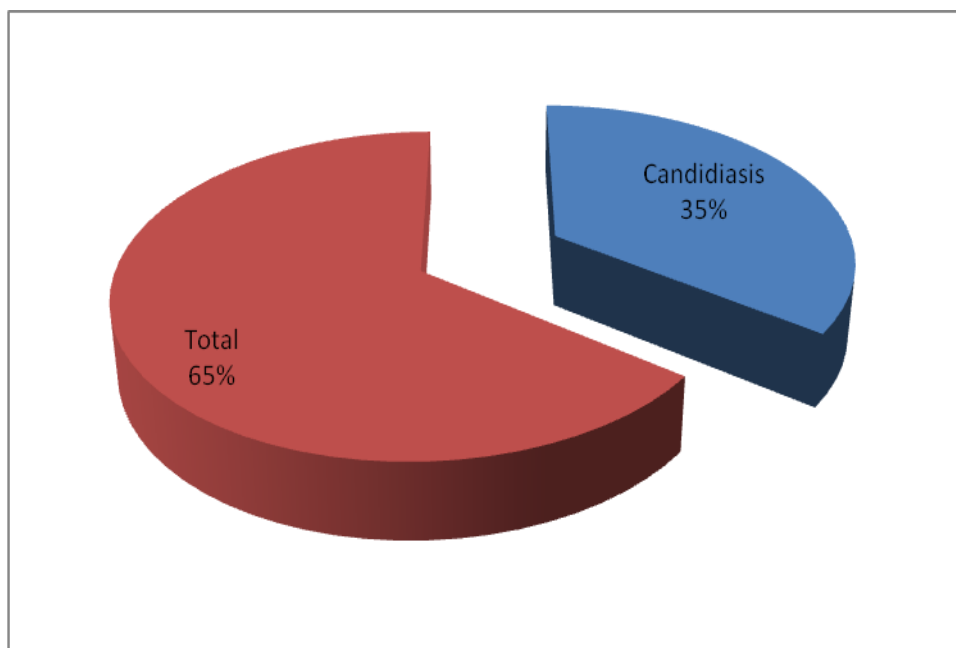

Figure 2: Prevalence of candidiasis among pregnant woman

Table 1: Age wise distribution of positive cases among study population

Age group (yrs)	Total (%)	Positive cases (%)
15-20	29 (18.47)	17 (19.10)
21-25	67 (42.67)	36 (40.44)
26-30	42 (26.75)	29 (32.58)
>30	19 (12.10)	7 (7.86)
Total	157	89

Frequency distribution of Candida Isolates

A total of 89 isolates were isolated in which 85 isolates were identified as candidal isolates and rest of them were not identified. Of 85 candidal isolates, 57 (64.04%) were *C. albicans,* 11 (12.35%) were *C. glabarata,* 9 (10.11%) were *C. dublenesis,* 5 (5.61%) were *C. tropicalis* and 3 (3.37%) were *C. krusei.* Among all candida isolates, *C. albicans* was found to be predominant organism to cause

candidiasis followed by *C. glabarata.* The results are shown in Figure 3.

Distribution of Candidiasis according to Gestational period

Most of the respondents had suffered from candidiasis in 2nd trimester of gestation period of 47 (55%) followed by 1st trimester with 29 (34.11%). The results are shown in Figure-4.
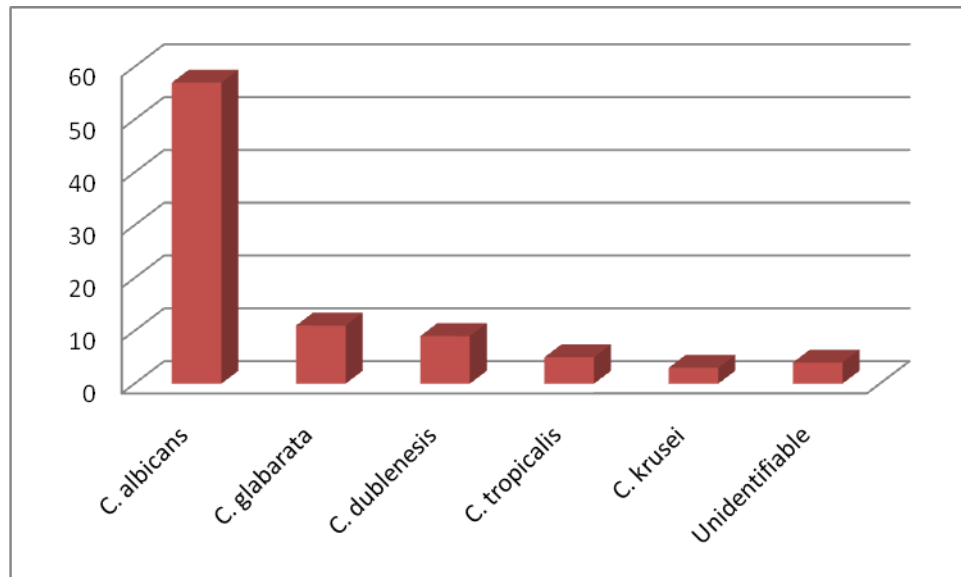

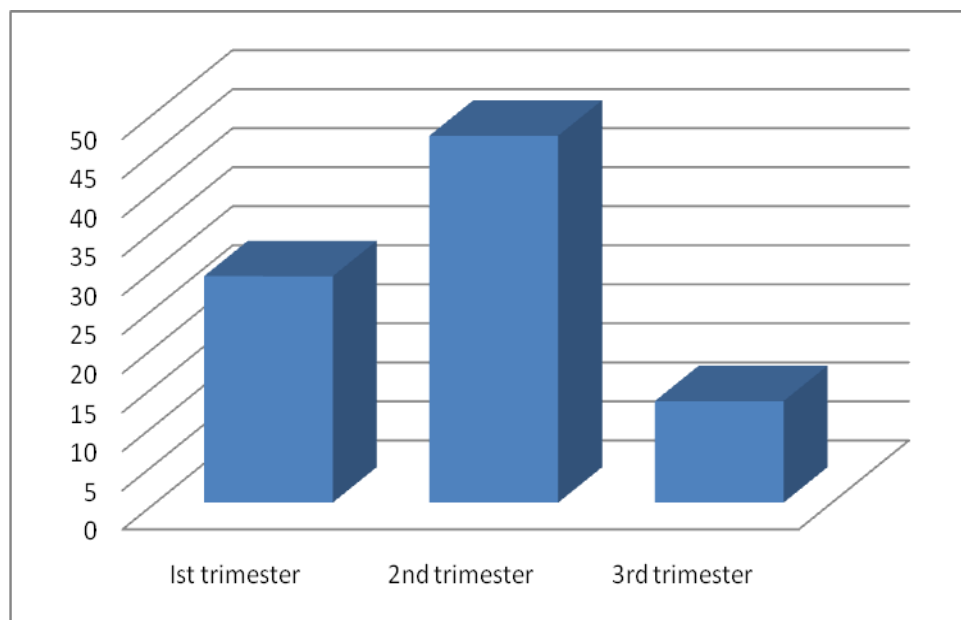**Figure 3:** Pattern of Candidal isolates

Figure 4: Candidiasis distribution in terms of gestation period

Pattern of Sign and Symptoms of Vaginal Candidiasis

The highest number of positive cases was observed in those of the respondents who had symptoms of vaginal candidiasis. The results was found to be statistically significant (p = 0.001) which are shown in Table 2.

Distribution of Risk factors among Study population

The highest number of positive cases was found in those respondents who had none of the below risk factors which was found to be statistically insignificant (p = 0.125). The results are shown in Table 3.

Educational and Occupational Distribution of Pregnant women with Positive cases

Most of the respondents had illiterate and unemployed of high positive cases with 35.95% and 44.94% followed by primary level and employed with 29.21% and 34.83% which was found to be statistically significant and insignificant respectively. The results are shown in Table 4.

Table 2: Frequency distribution of cases in terms of sign and symptoms

Sign & Symptoms	Positive Cases Number (%)	Negative Cases Number (%)	Total	p- value
Asymptomatic	22 (24.71)	41 (60.29)	63 (40.12)	
Symptomatic	67 (75.28)	27 (39.70)	94 (59.87)	**0.001**
Total	**89**	**68**	**157**	

Table 3: Risk factors distribution among study population

Risk Factors	Positive Cases (%)	Negative Cases (%)	Total (%)	p-value
Diabetes	6 (6.74)	1 (1.47)	7 (4.45)	
Previous Candidiasis	21 (23.59)	14 (20.58)	28 (17.83)	
Previous Antibiotic	19 (21.34)	9 (13.23)	35 (22.29)	
Oral Contraceptives	6 (6.74)	2 (2.94)	8 (5.09)	**0.125**
Previous Intrauterine	14 (15.73)	5 (7.35)	19 (12.10)	
HIV/ AIDS	1 (1.12)	0 (0)	1 (0.63)	
None	22 (24.71)	37 (54.41)	59 (37.57)	
Total	**89**	**68**	**157**	

Table 4: Distribution of respondents with positive cases in terms of Education level and Occupation

n=157

Educational level	Total no (%)	Positive Cases (%)	p-value
Illiterate	67 (42.67)	32 (35.95)	
Primary	42 (26.75)	26 (29.21)	**0.001**
Secondary	19 (12.10)	12 (13.48)	
Higher	29 (18.47)	19 (21.34)	
Occupation			
Unemployed	83 (52.86)	40 (44.94)	
Employed	41 (26.11)	31 (34.83)	**0.328**
Student	33 (21.01)	18 (20.22)	

DISCUSSION

Candidiasis is the most common opportunistic fungal infection and is responsible for 90% of the cases of infectious vaginitis (Hedayati and Shafiei, 2010; Adad *et al.,* 2001). Increased secretion of reproductive hormones both progesterones and estrogens during pregnancy favors the formation of infection. Progesterone has suppressive effects on the anti-candida activity of neutrophils (Adad *et al.,* 2001) while estrogen has been found to reduce the ability of the vaginal epithelial cells to inhibit the growth of Candida albicans and also decrease immunoglobulins in vaginal secretions resulting in increased vulnerability of pregnant women to vaginitis (Anorlu *et al.,* 2004).

This study reveals the prevalence of vulvovaginal candidiasis among pregnant women was found to be 35%. Nurat *et al.,* 2015 conducted the same study at LAUTECH Teaching Hospital in Ogbomoso and reported the prevalence of VVC was 25%. This prevalence rate is in tandem with 26% reported in Ibadan (Anorlu *et al.,* 2004) which is almost double the rate reported in Burkina Faso (14%). The differences could be due to geographic, ethnic and socioeconomic factors, as well as differences in sampling and culturing techniques. Variations may also reflect differences in sexual practice and environmental factors such as hygiene and nutrition (Hansen *et al.,* 2004; Busetti *et al.*, 2007). The prevalence reported in this study is higher than the 30.7% reported in Jamaica (Kamara *et al.,* 2000) and the rate of 30% reported in Nnewi, a town in Nigeria (Okonkwo and Umeanaeto, 2010) which shows the increasing trend of VVC in pregnancy. This high prevalence of vaginal candidiasis may lead to pregnancy complications like abortions, premature birth, low birth weight and other morbidities.

This study found the highest numbers of VVC were in 21-25 years age group (40.44%) followed by 26-30 years with 32.58% which is almost similar to the study reported by Nurat *et al.,* 2015. The age group contains women who are younger and are sexually active have low vaginal defense mechanisms against *Candida* species (Kent, 1991). They also have the habit of using contraceptives especially the emergency pills to prevent pregnancy. They also misuse drugs especially antibiotics for treatment of such infections. The frequency was also high within this age group in this study because of sexual promiscuity and the use of contraceptives that are predisposing factors of vaginal candidiasis. The misuse of drugs results to drug resistance especially to the common antifungal agents used for the treatment of vaginal candidiasis. This might have also contributed to the high frequency of the infection in this age group. This study also found as the increased in ages of women VVC was less prevalence. Women in the age group 41-45 years are close to their menopause and are less active sexually. They also rarely use contraceptives to prevent pregnancy and have increased vaginal immunity due to decreased levels of estrogens and corticoids.

The present study highlights that *C. albicans* (64.04%) was found to be predominant organism to cause VVC in pregnancy followed by *C. glabarata.* The results of this study was in accordance with Nurat *et al.,* 2015, Samuel *et al.,* 2015, Akortha *et al.,* 2009 and Nelson *et al.,* 2013. Pregnant women were more prone to Candidal infection. During pregnancy, which is listed as a risk factor, vagina is more sensitive, and the infections occur significantly more often. The high incidence of vaginitis in pregnant women is related to levels of estrogens, which is in turn considered the primary factor for the observed Vulvovaginal candidiasis was more prevalent in women (Sobel, 2012).

The hormonal environment of the vagina during pregnancy can enhance candida colonization and serve as risk factors. Progesterone has suppressive effect on the anti-candida activity of neutrophils while estrogen has been found to reduce the ability of vaginal epithelial cells to inhibit the growth of *Candida albicans* (Aslam *et al.,* 2008). Moreover, a large proportion of women with chronic recurrent candidiasis first present with the infection during pregnancy. In pregnant women, vaginal candidiasis has been related to emotional stress and suppression of immune system which steps up the risk of Candida species overgrowth and become pathogenic (Nelson *et al.,* 2013).

This present study revealed that most of the respondents had suffered from candidiasis in 2nd trimester of gestation period of 55% followed by 1st trimester with 34.11%. Nurat *et al.,* 2015 reported 54.3% prevalence of VVC was observed in the second trimester followed by 25.7% in first trimester and 20% in the third trimester. Deepa *et al.,* 2014 reported 54% prevalence in second trimester, 30% in third trimester and 16% in first trimester. The study conducted by Oyewol *et al.,* 2013 also observed the highest incidence of vaginal candidiasis among pregnant women in their second trimester (61%), which is almost in accordance with this study.

This study reflected the highest number of VVC was observed in those of the respondents who had symptoms of vaginal candidiasis and was found to be statistically significant (p = 0.001). A similar study was conducted by Kanagal *et al.,* 2014 reported 82% of candida positive women were symptomatic and the remaining 18% were asymptomatic which was statistically significant (p< 0.01), was in accordance with the present study.

The highest number of VVC was found in those respondents who had no any kinds of risk factors like diabetes, previous Candidiasis, previous antibiotic, oral contraceptives, previous intrauterine, HIV/ AIDS which was found to be statistically insignificant (p = 0.125). But Kanagal *et al.,* 2014 highlights 60% of pregnant women with vaginal candidiasis had risk factors like diabetes, previous candidiasis infection, use of antibiotics, oral contraceptive pills and intra uterine contraceptive devices which was statistically significant. This is not in concurred with this study (Kanagal *et al.,* 2014).

This study represented that most of the respondents had illiterate of high positive cases of VVC with 35.95% and 44.94% which was found to be statistically significant (p=0.001). Similar finding was also obtained in the study conducted by Shrestha *et al.,* 2011 which is in accordance with this study. This study also found the highest number of respondents were unemployed who had higher VVC and found to be statistically insignificant (p=0.328). The low economic status, lack of education, lack of a female consultant at the health service centre, hesitance to approach medical service, social cultural structure might be regarded as the cause of higher prevalence of vaginitis among less educated women.

CONCLUSION

The present study concluded that the prevalence of vulvovaginal candidiasis in the pregnant women was increasing from the previous study and found to be high especially within the age group of 21 to 25 years and in those women in their 2nd trimester of pregnancy. *Candida albicans* was the most prevalent vaginal *Candida* species isolated causing VVC in pregnant women. Non-albicans *Candida* species were also isolated which indicates their emergence as opportunistic pathogens in immuno-compromised patients. This study also concluded the higher incidence of vulvovaginal candidiasis in those pregnant women who had symptoms of VVC, who were illiterate and unemployed.

Early detection and early diagnosis may improve the clinical condition of the pregnant women. Classic medical treatment of VVC lies on the use of antifungal drugs such as Nystatin, Amphotericin B and Imidazoles which may also be associated with some serious side effects (Sanglard, 2002). Research has also shown an increasing resistance of fungi to imidazole derivatives. Therefore, appropriate treatment without any side effect should be given to culture positive women in order to prevent subsequent infection of the neonate and secondary infection to the mother. The need for proper and well coordinated sex education should be organized for pregnant women in order to prevent candidal infections which will be very imperative and desirous. Strict routine maternal health checks up should be conducted among all the pregnant women at regular interval of time for early diagnosis of candidiasis.

ACKNOWLEDGEMENT

REFERENCES

Adad SJ, De Lima RV, Sawan ZT, Silva ML, De Souza M.A, Saldanha JC, Falco VA, Da Cunha AH, Murta EF (2001). Frequency of *Trichomonas vaginalis*, *Candida sp* and *Gardnerella vaginalis* in cervical-vaginal smears in four different decades. Sao Paulo Med. J. 119(6): 200-205.

Akah PA, Nnamani CE, Nnamani PO (2010). Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. J. Med. Med. Sci. 1(10): 477-452.

Akinbami NA, Babalola OG, Shittu OM, Tijani MA and Adekola AS (2015). Detection and Epidemiology of Vulvovaginal Candidiasis among Asymptomatic Pregnant Women Attending a Tertiary Hospital in Ogbomoso, Nigeria. Int. J. Biomed. Res. 6(07): 18-23.

Akinbiyi AA, Watson R, Feyi-Waboso P (2008). Prevalence of Candida albicansand bacterial vaginosis in asymptomatic pregnant women in South Yorkshire, United Kingdom. Arch. Gynecol. Obstet. (278): 463-466.

Akortha E, Chinwe NO, Nwaugo VO (2009). Antifungal resistance among Candida species from patients with genitourinary tract infection isolated in Benin City, Edo State Nigeria. African J Microbio Res. 3(11): 694-699.

Alli JAO, Okonko IO, Odu NN, Kolade AF, Nwanze JC (2011). Detection and prevalence of Candida isolates among patients in Ibadan, Southwestern Nigeria. J. Microb. Biotechnol. Res. (1): 176-184.

Alo MN, Anyim C, Onyebuchi AK, Okonkwo EC (2012). Prevalence of asymptomatic Co Infection of Candidiasis and Vaginal Trichomoniasis among Pregnant Women in Abakaliki, South-Eastern Nigeria. J. Nat. Sci. Res. (2): 87-91.

Anorlu R, Imosemi D , Odunukwe N , Abudu O , Otuonye M (2004). Prevalence of HIV among women with vaginal discharge in a gynecological clinic. Natl. Med. Assoc. 96(3): 367–371.

Aslam M, Hafeez R, Ijaz S, Tahir M (2008). Vulvovaginal Candidiasis in Pregnancy. Biomedica (24): 54-56.

Azaz S, Chaudhary A, Kareem F (2005). Bacterial vaginosis in patients at MH Rawalpindi. Pak. Armed Forces Med. J. (55): 24-28.

Busetti MP, Agaro D and Campello C (2007). Group B streptococcus prevalence in pregnant women from North-Eastern Italy: advantages of a screening strategy based on direct plating plus broth enrichment. J. Clin. Pathol. (60): 1140-1143.

Butte NF (2000). Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Amer. J. Clin Nutr. (71): 1256-1261.

Cronje HS, Joubert G, Muir A, Chapman RD, Divan P, Barn RH (1994). Prevalence ofvaginitis, syphilis and HIV infection in women in the Orange Free State. S. Atr. Med. J. (84): 602-605.

Deepa VK, Vashe KV, Rashmi K, Harish S, Aparna R (2014). Prevalence of Vaginal Candidiasis in Pregnancy among Coastal South Indian Women. J. Womens Health, Issues Care. 3(6): 3-8.

Feyi-Waboso PA and Amadi AN (2001). The prevalence and pattern of vaginal candidiasis in pregnancy in Abia. J. Med. Invest. Prac. (2): 25-27.

Fidel P and Cutright CJ (2000). Effects of reproduction hormones on experimental vaginal candidiasis. Infect. and Immun. 68 (2): 651-657.

Gonzalez M, Elizondo M and Ayala J (2008). Trends in Species Distribution and Susceptibility of Blood stream Isolates of *Candida* collected in Monterrey Mexico to Seven Antifungal Agents, J. Clin. Microbiology. 46(9): 2902-2905.

Hansen SM, Uldbjerg N, Kilian M, Skov Sørensen UB (2004). Dynamics of *Streptococcus agalactiae* Colonization in Women during and after Pregnancy and in Their Infants. J. Clin. Microbiol. 42(1): 83-89.

Hedayati T and Shafiei G (2010). Candidiasis. eMedicine Specialties.

Kamara P, Hylton-Kong T, Brathwaite A, Del Rosario GR, Kristensen S (2000). Vaginal infections in pregnant women in Jamaica: prevalence and risk factors. Int. J. STD and AIDS. 11(8): 516-520.

Kanagal DV, Vineeth VK, Kundapur R, Shetty H (2014). Rajesh Prevalence of Vaginal Candidiasis in Pregnancy among Coastal South Indian Women. J. Women's Hea. Issues & Care. 3(6): 1-3.

Kent H (1991). Epidemiology of Vaginitis. American J. Obs. Gynec. 165(4): 1168-1176. http://dx.doi.org/10.1016/S0002-9378(12)90722-X

Leon ED, Jacober SJ, Sobel JD, Foxman B (2002). Prevalence and risk factors for vaginal Candida colonization in women with type 1 and type 2 diabetes. BMC Infect. Dis. 2(1):1-6.

Mitchell H (2004). Vaginal Discharge-Causes, Diagnosis and Treatment. Biomed. J. (328): 1306-1308.

Nelson M, Wanjiru W, Margaret MW (2013). Prevalence of Vaginal Candidiasis and Determination of the Occurrence of Candida Species in Pregnant Women Attending the Antenatal Clinic of Thika District Hospital, Kenya. Open J. of Med. Microbio. (3): 264-272.

Nviriesy P (2008). Vulvovaginal Candidiasis and bacterial vaginosis infections. North Am. J. Clin. Dis. (22): 637-652.

Ohmit S , Sobel J , Schuman P, Duerr A, Mayer K, Rompalo A (2003). HIV Epidemiology Research Study (HERS) Group. Longitudinal study of mucosal Candida species colonization and candidiasis among human immunodeficiency virus (HIV)-seropositive and at-risk HIV seronegative women. J. Infect. Dis. (188): 118–127.

Okonkwo NJ and Umeanaeto PU (2010). Prevalence of Vaginal Candidiasis among Pregnant Women in Nnewi Town of Anambra State, Nigeria. Int. Multi Disciplinary J. Ethiopia 4(4): 539-548.

Oyewole OA, Okoliegbe IN, Alkhalil S, Isah P (2013). Prevalence of Vaginal Candidiasis among Pregnant Women Attending Federal University of Technology, Minna, Nigeria, Bosso Clinic Res. J. Pharm. Biol. Chem. Sci. (4): 113-120.

Parveen N, Munir AA, Din I, Majeed R (2008). Frequency of vaginal candidiasis in pregnant women attending routine antenatal clinic. J. Coll. Physicians Surg. Pak. (18): 154-157.

Prakash S and Yadav K (2015). Maternal Anemia in Pregnancy: An Overview. Int. J. Phar. Pharm. Res. 4(3): 1-16.

Prakash S, Yadav K, Bhardwaj B, Chaudhary S (2015). Incidence of Anemia and its Socio-demographic determinants among pregnant women attending for antenatal care: A cross sectional study. Int. J. Med. Health Res. 1(3): 12-17.

Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P (2012). Epidemiologic Features of Vulvovaginal Candidiasis among Reproductive- Age Women in India, Hindawi Publishing Corporation. Infect. Dis. Obstet. Gynecol. (8): 42-45.

Reed BD, Zazove P, Pierson CL, Gorenflo DW, Honocks J (2003). Candida transmission and sexual behaviours as risks for a repeat episode of candida vulvovaginitis. J. Women Heal. 2(10): 979-989.

Richardson M and Warnock D (2003). Fungal Infection, Diagnosis and Treatment, 3rd Edition, Blackwell Publishing, London. pp. 38-43.

Samuel1 O, Ifeanyi O , Ugochukwu1 O (2015). Prevalence of Candida Species among Vaginitis Symptomatic Pregnant Women Attending Ante-natal Clinic of Anambra State University Teaching Hospital, Awka, Nigeria.   Bioengineering and Biosci. 3(2). 23-27.

Sanglard FCD (2002). Odds. Resistance of Candida species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect. Dis. 2: 73.

Shrestha S, Tuladhar NR, Basnyat S, Acharya GP, Shrestha P, and Kumar P (2011). Prevalence of vaginitis among pregnant women attending Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. Nepal Med. Coll. J. 13(4): 293-296.

Singh SI (2003). Treatment of Vulvovaginal Candidiasis. Clin. J. Rev. 136(9): 26-30.

Sobel JD (1985). Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. Am. J. Gynecol. (152): 924- 935.

Sobel JD (1997). Vaginitis, The New England Journal of medicine. (337): 1896-1903.

Sobel JD (2003). Management of patients with recurrent vulvovaginal candidiasis, Drugs. (63): 1059–1066.

Sobel JD (2012). Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis, Am. J. Obstet. Gynecol. 7(3): 5-9.