CHAPTER-I

1 INTRODUCTION

Vaginitis is the medical term used to refer any infection or inflammation of the vagina, most often caused by bacteria, fungi, or parasites. Vaginal discharge is the sticky mucus that is produced by the vagina. The cause is usually a change in the normal balance of vaginal bacteria or an infection. Vaginal symptoms are one of the most common reasons for gynecological consultation accounting for approximately 10 million office visits each year. Although vaginitis can have a variety of causes, most often it is associated with infection or atrophic changes (Owen and Clenney, 2004). Vaginitis can also result from reduced estrogen levels after menopause for which a microbiological diagnosis is sought (Gillespie, 1994) which may be associated with sexually transmitted diseases (STDs) and causes different types of morbidity in women specially of reproductive age group (Aryal, 1997). Most vaginal symptoms are not signs of serious disease such as cancer or AIDS, and the majority of such complaints are not due to STDs.

Vaginal mucous glands make small amounts of fluid and flows out of the vagina each day, carrying out old and dead mucus cells that have lined the vagina and also lactobacilli. This is the body's way of keeping the vagina healthy and clean. The discharge is usually clear or milky with no malodor. Women always have some vaginal discharge that changes through out the month due to hormonal changes and the composition of vaginal flora changes with age, stress, hormonal influence, general health status, and sexual activity.

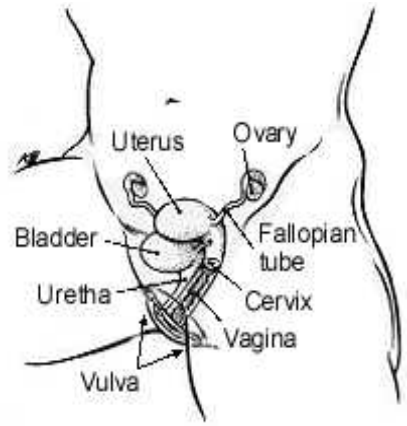


Figure 1 Anatomy of female reproductive tract

All patients with the history of white discharge may not have sexually transmitted infection (STI) but should be investigated for STI. Abnormal vaginal discharge changes in quantity,

color and odor compared with normal vaginal discharge and irritation or itching or burning of the vagina or cervix indicate infection. In some cases, vaginitis results from organisms that are passed between sexual partners.

The commensal flora of the vagina varies with age. Before puberty, when the secretion is not acidic (pH 6.5-7.5), the flora mainly consists of Staphylococci, Streptococci, other than *Streptococcus pyogenes*, Diptheroid bacilli and Coliform bacilli. From the puberty to the menopause, the secretion is acidic (pH 4.5) and lactobacilli predominates and other organisms are present in smaller number. After the menopause the secretion is again non-acidic and Lactobacilli no longer predominate as the flora in the vaginal discharge (Madigan *et al*, 2003).

The three most common infectious forms of vaginitis are bacterial vaginosis, vaginal candidiasis, and trichomoniasis **(**Sobel, 1997).The appearance or characteristic of vaginal discharge differs from the type of vaginal infection such as *Trichomanas* spp., *Candida* spp., and BV. *Trichomonas vaginalis* usually produces frothy yellow (or yellow-green), in BV the discharge is grey/white, thin, and watery with foul and fishy odor whereas the discharge in candidiasis appears typically thicker and curd like. In United states BV accounts for 40-50% of vaginitis cases, candidiasis for 20-25% and trichomoniasis for 15-20%.

T. vaginalis is a protozoan parasite that causes trichomoniasis. Trichomoniasis usually spreads through sexual contact (Greenwood *et al*, 1997). The organism is not a normal inhabitant of the vagina. The parasite can survive for several hours in a moist environment. In theory, a person could get the infection by having direct contact with surfaces that are contaminated with the parasite, like a wet towel, a bathing suit, or a toilet seat.

A yeast infection (candidiasis) occurs when there is an over abundance of yeast, mostly the *C. albican*, often caused by a change in the pH balance of the vagina due to recurrent yeast infections or recent antibiotic treatment. It is common in women of childbearing age usually

not contracted from a sexual partner. Seventy-five percent of all women have one episode of candidiasis in their lifetime (Monif, 1985).

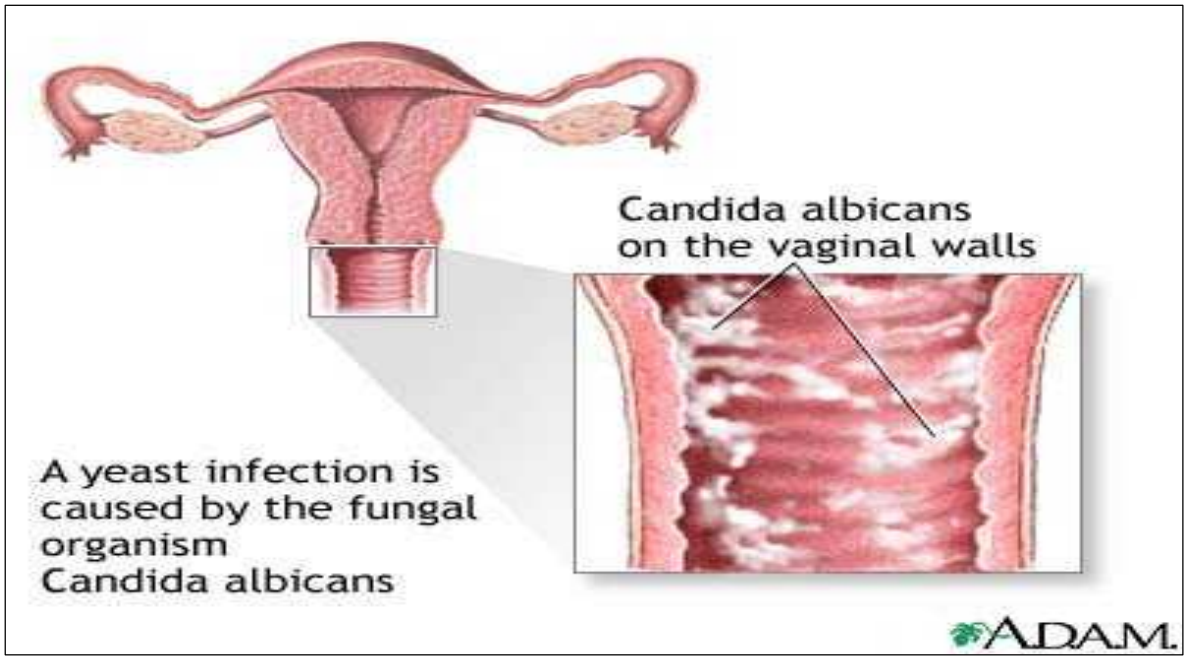


Figure 2 Candidiasis on the vaginal wall

Bacterial vaginosis is a condition characterised by raised vaginal pH and milky discharge in which the normal vaginal flora is replaced by a mixed flora of aerobic, anaerobic and microaerophilic species (Hill *et al*, 1985). *Gardnerella vaginalis is* the common cause of BV as these organisms do not invade the subepithelial tissue (thus is termed as vaginosis rather than vaginitis). Recurrence of BV is common and can coexist with other vaginal infections. One third to three quarters of affected women are asymptomatic (Sobel, 1990).

Although, the problem seems small but for the sufferer it is the matter of immense physical and psychological problem that may require instant attention, which is lacking in the most of the hospitals and clinics. Reasons other than women delay in seeking medical care include lack of time, poor health awareness, stigma attach to reproductive infections and a culture of silent endurance. In Nepal, very few studies have been implicated in the prevalence and therapeutic consideration of vaginitis. So it is hard to know how frequent this disease is among Nepalese pregnant women. It has been reported that one in three women wants consultation for vaginal discharge (Pradhan, 2001).

Vaginitis may result in sterility, abortion, and stillbirth. Since the disease leads to many complications, it is of the prime importance to know the causative organism and prevalence of the disease among Nepalese pregnant women. This implies the main aim of this study.

The married women are reluctant to seek medical treatment because of the lack of privacy, lack of a female doctor at the health facility, the cost of treatment and their subordinate social status. A culture of silence shrouds gynecologic morbidity throughout India and elsewhere. The findings of this study may be helpful to the people and professionals for the prevention/control and treatment of vaginitis and prevent from stillbirth, abortion, and sterility. Many women think their symptoms are normal occurrence or are reluctant to mention those (Prasad *et al*, 2005). This study also helps to find the prevalence of vaginitis in the pregnant women, to disseminate knowledge regarding vaginitis as many are unaware of it, to fulfill the gap in research carried out in the field of vaginitis, and to focus the main cause of vaginitis and to make women aware of it so as to prevent from reinfection in future.

Bacterial vaginosis (BV) may be a cofactor for HIV transmission, especially among younger women. Young women are particularly susceptible to STIs because they have fewer antibodies to fight against pathogens (Prasad *et al*, 2005). BV has been linked to preterm birth, post operative infections, pelvic inflammatory disease, infertility and acquisition of HIV and genital herpes simplex infections (Cherpes *et al*, 2003). Thus, this study is hoped to help to prevent and cure such effects.

CHAPTER-II

2 OBEJECTIVES

2.1 General objective

- To study the prevalence of common types of vaginitis (candidiasis, trichomoniasis and bacterial vaginosis) of the pregnant women visiting “Thapathali Maternity Hospital, Thapathali”, Kathmandu, Nepal.

2.2 Specific objectives

- To reveal the prevalence of vaginitis among the pregnant women visiting Thapathali Maternity Hospital, Nepal.
- To know the etiological agents of vaginitis among the pregnant women studied.
- To determine the prevalence of trichomoniasis, vaginal candidiasis and bacterial vaginosis.
- To study the vaginitis among the pregnant women of different settlement areas.
- To study the vaginitis among the pregnant women of different ethnic groups.
- To asses the most prevalent age group of vaginitis in pregnancy state.
- To study the vaginitis among the pregnant women involved in different occupation.
- To study the vaginitis in symptomatic and asymptomatic cases among pregnant women.
- To study the prevalence of types of vaginal infection in pregnant women having vaginitis.

CHAPTER-III

3 LITERATURE REVIEW

3.1 Physiology and ecology of the vagina

Qualitative and quantitative studies in humans have confirmed that the vaginal flora is a dynamic and closely interrelated system. Lactobacilli are most prevalent but many other facultative and anaerobic organisms are also present. An inverse relationship exists between the concerntration of lactobacilli and other bacterias such as anaerobes. Aerobic bacterias decrease premenstrually whereas anaerobes, in general, remain at constant levels. During menarche, pregnancy, postpartum period, menopause, and post-operative trauma, dramatic changes occur in the microbial flora. The role of an IUCD and OD in causing changes in the microbial flora is under great secruting at the present time. Overall, disturbances in the vaginal ecosystems may have a potential impact on many diseases, and thus deserve careful studies (Paavonen, 1983).

3.2 Vaginal discharge

Changes in the activity of the vaginal epithelium and the vaginal secretion, which occur at different times during a woman's life, has profound influence on the defense against vaginal infection. In the adult, the normal vaginal moisture or secretion consists of vaginal transudate containing desquamated vaginal epithelial cells, which may give it a creamy color, mucus secreted by cervical glands and to a small extent, secretion from the endometrial glands. Its viscosity depends mainly on the cervical component. Estrogens and sexual stimulation are examples of factors, which increase vaginal fluid. Major organic constituents of the vaginal fluid are proteins, carbohydrates, and fatty acids. Organic acids arise as metabolic byproducts of vaginal bacterial flora, cause the vaginal odor, and show cyclic changes except in oral contraceptive users.

There is a cyclic variation in the amount of secretion; it is heavier premenstrually and there is an increased secretion of clear cervical mucus at time of ovulation. There are no glands in vagina and transudate passes through the stratified epithelium. The epithelium contains

glycogen, which is converted to lactic acid by Dodelein's bacilli, which are normally present in the vagina. The discharge is usually less profuse and may be masked more easily in the female by vaginal secretions, thus the presence of infection is more likely to be asymptomatic in females (Baron *et al*, 1994).

During pregnancy many women notice a significant change in the amount of vaginal discharge that their bodies produce. This is due to the increased estrogen and blood flow that body needs during pregnancy. As the due date approaches, it may be noticed that the body produces even more vaginal discharge. These extra secretions will help to facilitate the baby's move down the birth canal. It may also be noticed that the discharge becomes increasingly thin and runny towards end of the pregnancy. This is usually a sign that the mucus plug covering the cervix is beginning to dislodge, and labor is not far away. The pregnant women are most susceptible to different infections.

3.2.1 Normal vaginal discharge

All women have some vaginal discharge. Normal discharge may appear clear, cloudy white, and/or yellowish when dry on clothing. It may also contain white flecks and at times may be thin and stringy. Changes in normal discharge can occur in many reasons, including menstrual cycle, emotional stress, nutritional status, pregnancy, usage of medication including birth control pills, and sexual arousal.

Generally, there is a small amount of clear mucus discharge, which starts from the first week of the menstrual period increasing in quantity and getting thicker close to the time of ovulation. Most women begin producing vaginal discharge during puberty and will continue to produce about 4 milliliter of discharge every day for the rest of their lives. Many things can disturb the balance of a healthy vagina, including douching, feminine hygiene sprays, certain soaps or bubble baths, antibiotics, diabetes, pregnant or infections (Kent, 1991).

3.2.1.1 Role of lactobacilli as a protector against vaginitis (Soledad *et al*,1998)

Among the properties of most vaginal lactobacilli strains found in the vagina is their ability to release hydrogen peroxide (H_2O_2) in appreciable amounts *in vitro*. H_2O_2 can be auto

inhibitory or toxic to adjacent bacteria, fungi, viruses, or mammalian cells, particularly in the presence of peroxidase-mediated antimicrobial system *in vitro*. Lactobacilli are believed to interfere many pathogens by different mechanisms.

1. The first is competitive exclusion of genitourinary pathogens from receptors present on the surface of the genitourinary epithelium.
2. Second, lactobacilli coaggregate with some uropathogenic bacteria, a process that, when linked to the production of antimicrobial compounds, such as lactic acid, hydrogen peroxide, bacterocin-like substances, and possibly bio-surfactants, would result in inhibition of the growth of the pathogen.
3. Competition with exogenous bacteria for adherence receptor sites on vaginal epithelial cells.
4. Elaboration of antimicrobial-like substances, bacteriocins.
5. Co-aggregation with bacteria, and stimulation of the superficial vaginal immune system to enhance local defense mechanisms against non-resident bacteria.

3.2.1.2 Vaginal discharge in pregnancy and the postpartum period

Vaginal discharge as a symptom or sign of reproductive tract infection presents different challenges during pregnancy, because physiological changes during pregnancy can affect the normal microbiological environment (flora) of the vagina. For example, discharge may be more abundant and yeast infection is more common. Women with vaginal discharge should be carefully questioned and examined to make sure that the discharge is not an early sign of a more serious problem. For example,

- In early pregnancy, discharge may mask spotting or light bleeding that could indicate ectopic pregnancy, threatened abortion, or cervical cancer.
- A watery discharge in late pregnancy could be amniotic fluid from ruptured membranes.
- A careful history and examination will usually provide clues that will help distinguish simple vaginitis from more serious conditions. When discharge is accompanied by bleeding, fever, abdominal pain or amniotic fluid leakage, the patient should be managed or referred for possible sepsis.

- If pregnancy complications have been ruled out, all women with vaginal discharge should be treated for bacterial vaginosis, trichomoniasis and yeast infection. Yeast infection is very common during pregnancy and is often recurrent, so if a woman comes back with the same symptoms, she should be treated for yeast infection only.

3.2.2 Abnormal vaginal discharge

A small amount of vaginal discharge is usually normal. The discharge consists of secretions (mucus) produced mainly by the cervix but also in the vagina. The discharge is usually thin and clear, milky white, or yellowish. Its amount and appearance vary with age. Newborn girls normally have a vaginal discharge of mucus, often mixed with a small amount of blood. This discharge is due to estrogen absorbed from the mother before birth. It usually stops within 2 weeks, as the level of estrogen in the blood decreases. Normally, older infants and girls, except those near puberty, do not have any significant vaginal discharge.

During a woman's reproductive years, the amount and appearance of the normal vaginal discharge vary with the menstrual cycle. For example, at the middle of the cycle (at ovulation), more mucus is usually produced and the mucus is thinner. After menopause, the estrogen level decreases, often reducing the amount of normal discharge.

A vaginal discharge is considered abnormal if it is

- heavier than usual
- thicker than usual
- pus like
- white and clumpy like cottage cheese
- grayish, greenish, yellowish, or blood-tinged
- foul-smelling, fishy
- accompanied by itching, burning, a rash, or soreness

It's important to keep an eye out for changes in vaginal fluids. The following changes may indicate a problem

- change in odor, especially an unpleasant odor
- change in color or texture, especially greenish, grayish, or anything looking like pus
- vaginal itching, burning, swelling, or redness
- change in color that is caused by vaginal bleeding
- pain during intercourse
- painful urination
- light vaginal bleeding

3.2.3 Appearance of vaginal discharge in *Candida, Trichomonas* and *Gardnerella* infections

T. vaginalis: yellow-green purulent discharge with pH over 4.5

C. albicans: white discharge with pH below 4.5*

G. vaginalis: grey offensive, thin discharge with pH over 4.5*

*The normal reaction of vaginal discharge (puberty to menopause) is pH 3.0-3.5 (Cheesbrough, 2000)

3.5 Diagnostic criteria

Table 1 Diagnostic criterion of vaginal infections

Diagnosis	Diagnostic criteria
Trichomoniasis	Positive culture of viable *T. vaginalis* or positive wet mount preparation test
Bacterial vaginosis	Presence of at least three of the following: a)watery vaginal discharge, b) elevated pH(>6), c) positive amine odor test, d) presence of clue cells in Gram-stained vaginal smear
Vaginal candidiasis	Positive culture for *Candida* with the presence of clinical signs (red, inflamed tissue and curdy white discharge)

(Source: Helen *et al*, 2005)

3.6 Bacterial vaginosis

Bacterial vaginosis (BV) was first reported in 1995 by Gardner and Dukes, who described the unique clinical signs and symptoms and the distinctive nature of the vaginal discharge associated with it. They also described a "new" causative organism, which they named *Corynebacterium vaginale* later named *Haemophilus vaginalis* and subsequently renamed *G. vaginalis*. BV is currently the most prevalent cause of infectious vaginitis among women attending for genitourinary diseases (Gonzalez-Pedraza *et al*, 1999). BV, can be included under a number of names, e.g. anaerobic vaginosis and non-specific vaginosis, is a common condition (Gillespie, 1994). Originally termed 'non-specific vaginitis' to differentiate it from the 'specific vaginitis', trichomoniasis and candidiasis was long believed to represent infection by microorganism. Current research suggests that BV is actually not an infection but as a condition resulting from an imbalance in the vaginal flora (Pradhan, 2003).

Bacterial vaginosis is the most common cause of vaginitis during the childbearing years (Uma *et al*, 2006). BV accounts for 10 to 30 percent of cases of infectious vaginitis in women of childbearing age (Owen and Clenney, 2004). 40-50% of vaginitis cases are caused by BV. The occurrence of BV is difficult to determine, but studies have proposed that 10-41% of women have had it at least once. The occurrence of BV in the United States is highest among African-American women and women who have had multiple sexual partners and is lowest among Asian women and women with no history of sexual contact with men. BV is not considered a sexually transmitted disease although it can be acquired through sexual intercourse (Sorvillo *et al*, 1998). Even though higher rates of BV have been reported in sexually transmitted disease clinics and in women with multiple sexual partners, the role of sexual transmission is unclear. Studies indicate that treating the male sexual partner of a woman with BV is not beneficial and that even women who are not sexually active can have the infection (Barbone *et al*, 1990).

The average incidence of BV varies from 10-35% in patients visiting gynaecological wards, 10-30% in patients visiting obstetric wards and 20-60% in patients visiting services of sexually transmitted diseases. More than 50% of all women with BV are asymptomatic

(Georgijevic *et al*, 2000). BV has been found in 15 to 19 percent of ambulatory gynecology patients, 10 to 30 percent of pregnant patients and 24 to 40 percent of patients in sexually transmitted disease clinics (Bump and Buesching, 1988).

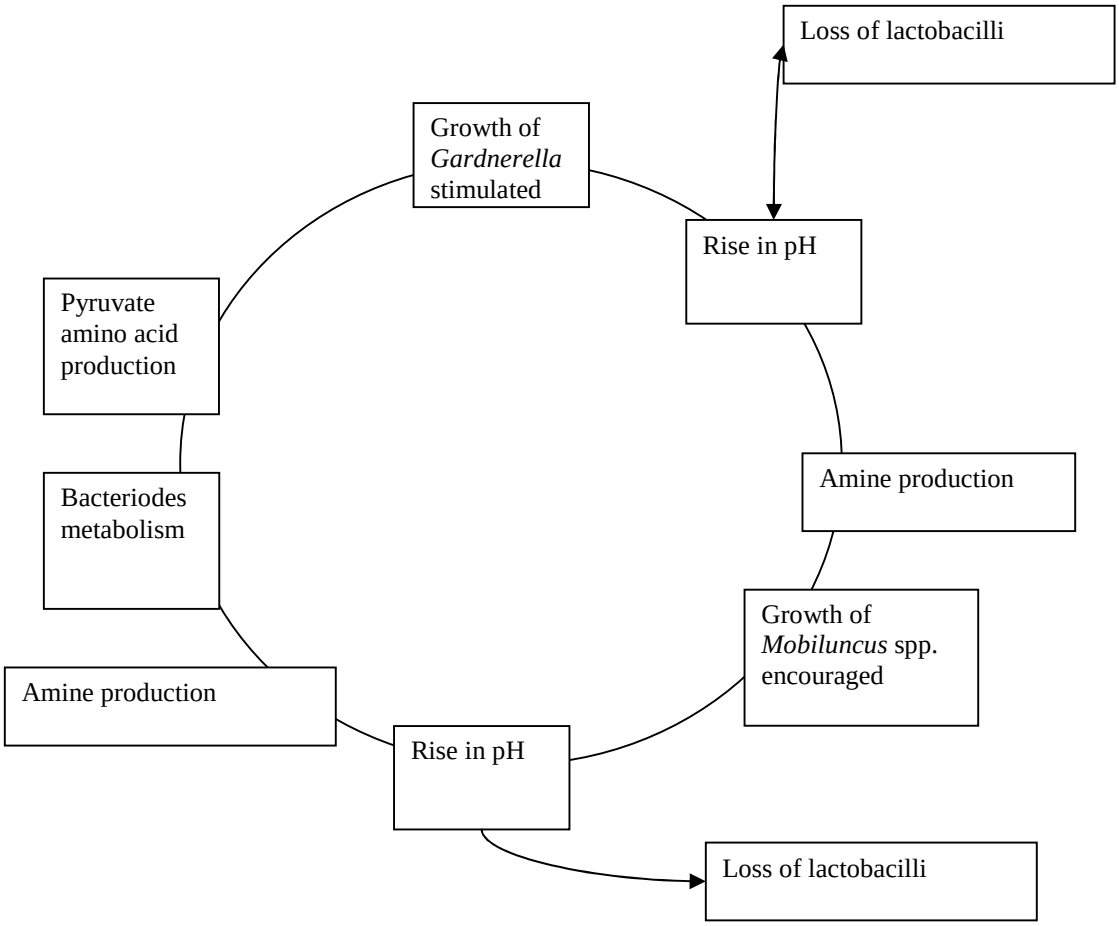


Figure 3 Showing production of amines by *Bacteroides* spp. lowers the pH stimulating growth of *Mobiluncus* spp. (pH >5.0) and *G. vaginalis* (pH >6.5). Amino acids and pyruvate produced by *Gardnerella* are metabolized by *Bacteroides* to amines. (Source: Gillespie, 1994)

Bacterial vaginosis is not caused by a particular organism but by a change in the balance of normal vaginal bacteria or by a change in the pH balance. Ninety percent of the bacteria found in a healthy vagina belong to the genus *Lactobacillus*. For various reasons, there is a shift in the bacterial population that results in overgrowth of other bacteria. Patients suffering from BV have very high numbers of such bacteria as *G. vaginalis, M. hominis, Bacteroides* spp. and *Mobiluncus* spp. and these bacteria can be found at numbers 100-

1,000 times greater than are found in the healthy vagina. In contrast, *Lactobacillus* spp. is very low in number or completely absent from the vagina of women with BV (Patricia, 2005). The absence of lactobacilli on Gram stain is another sign of BV (Baron *et al,* 1994).

The way in which the organisms interact to produce BV is not clear. *Gardnerella* has a pH optimum of 6-6.5 and the growth of *Mobiluncus* is favoured when the pH is greater than 5.0. The vaginal pH is usually kept very low due to acid production by commensal lactobacilli. It is thought that amine produced by the metabolism of *Bacteroides* raises the pH of the vagina sufficiently for *Gardnerella* and *Mobiluncus* to grow and cause symptoms (Gillespie, 1994). Although BV will sometimes clear up without treatment, all women with symptoms of BV should be treated to avoid such complications as PID. Male partners generally do not need to be treated. However, BV may spread between female sex partners. Treatment is especially important for pregnant women. All pregnant women who have ever had a premature delivery or low birth weight baby should be considered for a BV examination, regardless of symptoms and should be treated if they have BV. All pregnant women who have symptoms of BV should be checked and treated (Hillier and Holmes, 1999).

3.6.1 Signs and symptoms of bacterial vaginosis

- Increased amount of discharge
- Grey/white, thin, watery discharge
- Foul/fishy odor with discharge
- Increased odor to discharge immediately after intercourse

Nearly half of the women with bacterial vaginosis are asymptomatic.

3.6.2 Factors that increase the risk of BV (Georgijevic *et al,* 2000)

- Multiple partners,
- Exposure to semen,
- Prior trichomoniasis,

- Intrauterine device usage,
- Smoking,
- Indigent population and
- Frequent use of scented soap

The use of vaginal pH, amine whiff testing, wet mount and Gram's stain examination of vaginal discharge in some combination has been used in diagnostic sets of criteria that have been used for diagnosis of vaginitis (Sharma and Anand, 2003). The presence of small gram-negative rods or gram-variable rods and the absence of longer lactobacilli on a Gram stain of the vaginal discharge also are highly predictive of BV. However, this method of diagnosis is impractical in most of clinics and health center (Owen and Clenney, 2004).

3.7 Vaginal candidiasis

Candida vulvovaginitis also has been called vulvo-vaginal candidiasis, candidal vaginitis, monilial infection, or vaginal yeast infection**.** Although *C. albican* frequently is the cause of vaginal yeast infections, the organism can be present in asymptomatic women. Physicians also must remember that vaginal yeast infections may be caused by species other than *C. albicans*, such as *C. glabrata* and *C. tropicalis*. Infections with these species are less common than C. *albicans* infection and tend to be more resistant to treatment (Owen and Clenney, 2004). 20 to 25% of the vaginitis cases are Candida vulvovaginitis. It has been estimated that about 75% of all women get a vaginal yeast infection at least once. In 80% to 95% of the cases, candida vulvovaginitis is caused by an overgrowth of the yeast *C. albican*. It is not known what causes the yeast overgrowth. However, it is known that antibiotics can inadvertently kill normal bacteria in the vagina and cause an overgrowth of *Candida* spp. (Patricia, 2005).

During the reproductive years of a healthy woman's life, the vagina maintains a moist environment that is in constant fluctuation. The secretion of an alkaline transudate from the vaginal epithelium and cervical glands maintains this moist environment with a pH ranging from 3.8-4.5. In addition, the vagina and its microflora form a unique balanced environment that can change under pressure from external stimuli but returns to normal with removal of

the stimuli. It can vary in degree during the menstrual cycle, pregnancy, and sexual activity (Sobel *et al*, 1998).

Candida vulvovaginitis is not considered a sexually transmitted disease because *Candida* spp. is commonly found in the healthy vagina. It is rare to find this disease in girls before puberty and in celibate women. Symptoms appear when the balance between the normal microorganisms of the vagina is lost and the *C. albicans* population becomes larger in relation to the other microorganism populations. The occurrence of four or more attacks per year is called recurrent vaginal candidiasis (Patricia, 2005). Candidal vaginitis is common infection during pregnancy.

3.7.1 Some factors that may increase susceptibility to yeast infections

(Source: Greenwood *et al*, 1997)

- Increased stress
- Endocrine disease (e.g Diabetes mellitus)
- Use of oral contraceptive/ taking birth control pills
- Use of antibiotics (protective bacteria are destroyed by antibiotics, allowing yeast overgrowth)
- HIV infection/ acquired immunodeficiency syndrome

  (Owen and Clenney, 2004)
- Natural receptive states (infancy, old age, pregnancy)
- Neutropenia (primary or secondary to disease or immunosupression)
- Miscellaneous conditions (e.g zinc or iron deficiency)
- Poor ventilation in the genital area

3.7.2 Signs and symptoms of vaginal candidiasis

- Increased amount of discharge
- Redness, itching, burning in vaginal/vulvar area
- White, clumpy (cottage cheese-like), discharge
- Inflammation of the vulvar skin

- Pain with intercourse
- Urination, painful

3.7.3 Pathogenesis of candidiasis

During pregnancy, the vagina shows an increased susceptibility to infection by *Candida*, resulting in both a higher prevalence of vaginal colonization and a higher rate of symptomatic vaginitis (White and Lansen, 1997). The rate of symptomatic vaginitis is maximally increased in 3rd trimester and symptomatic reoccurrences are also more common during pregnancy (Sobel, 1995). High levels of reproductive harmones provides an excellent carbon source for *Candida* spp. by providing a higher glycogen content in the vaginal tissues. A more complex mechanism is likely, in that estrogen enhances adherence of yeast cells to the vaginal mucosa. A cytosol receptor or binding system for female reproductive hormones has been documented in *C. albicans. S*everal investigators demonstrated *in vitro* binding of female sex hormones to *Candida* spp. as well as the capicity of certain hormones to enhance yeast mycelia formation and hence virulence (Zhao *et al,* 1995). Not surprisingly, therefore rates of cure of Candidal vaginitis is significantly lower during pregnancy.

3.7.4 *Candida* adherence phenomena, from commensalisms to pathogenicity

Molecules present in the most external layers of *Candida* cells are essential for the adherence to host surfaces, playing a pivotal role in the pathophysiology of candidiasis. Receptors for fibrinogen, fibronectin and other components of the extracellular matrix have been described in *Candida* surfaces. Their expression may be influenced by particular host conditions, and these changes may be important in the transition from commensalisms to pathogenicity. Surface proteins are also essential in the interactions of the fungal cell with the various constitutive, inducible defense host mechanism that act during candidiasis (Senet, 1998).

The yeast *C. albicans* is an opportunistic fungal pathogen that is capable of inducing a range of superficial and systemic diseases in the immunocompromised host. Although it displays

a variety of virulence factors, one- the ability to adhere to host tissue is considered essential in the early stages of colonization and tissue invasion. Adherence is achieved by a combination of specific (ligand receptor interactions) and non-specific (electrostatic charge, van der waals forces) mechanisms, which allow the yeast to attach to a wide range of tissue types and inanimate surfaces (Cotter and Kavanagh, 2000).

3.8 Trichomoniasis

Trichomoniasis is primarily an infection of the genitourinary tract; the vagina is the most common site of infection in women. Trichomoniasis is the third most common cause of vaginitis (Egan and Lipsky, 2000). Trichomoniasis is one of the most common STDs in the United States. It is generally contracted after unprotected sex with an infected person. Trichomoniasis is caused by a tiny parasite, *T. vaginalis*, which infects the urinary tract. If left untreated it can cause serious damage to the reproductive tract. *T. vaginalis* may be emerging as one of the most important cofactors in amplifying HIV transmission, particularly in African-American communities of the United States. In a person co-infected with HIV, the pathology induced by *T. vaginalis* infection can increase HIV shedding. Trichomonal infection may also act to expand the portal of entry for HIV in an HIV-negative person. Studies from Africa have suggested that *T. vaginalis* infection may increase the rate of HIV transmission by approximately two fold (Sorvillo *et al,* 2001).

Trichomoniasis, which is sometimes called "trich," accounts for 15-20% of the cases of vaginitis. It is estimated that two million to three million American women get trichomoniasis each year (Patricia, 2005). People at highest risk to get this infection are individuals with multiple sex partners and with inadequate standards of personal hygiene (Ichhupujani and Bhatia, 1998). Nongonococcal urethritis may also be caused by infections with the protozoan *T. vaginalis.* The infection is more common in female; surveys indicate that 25 to 50% of sexually active women are infected, while only about 5% of men are infected (Madigan *et al*, 2003). Female infection may also be asymptomatic in up to 50% of patients. In remainder, it causes vaginal discharge that often has a very offensive odour and may be yellow/green and frothy (Gillespie, 1994). The organism is responsible for a mild

vaginitis, with discharge, which ordinarily responds to treatment with metronidazole or tinidazole (Greenwood *et al*, 1997).

3.8.1 Signs and symptoms of trichomoniasis

Symptoms of trichomoniasis can appear as early as 4 days after sex with an infected partner. But trichomoniasis often goes undiagnosed because symptoms may not appear until years later, if at all. In females, the symptoms of this infection include

- abundant and frothy vaginal discharge ranging in color from grey to green to yellow, with a watery to milky consistency
- foul odor
- itching and tenderness in or around the vagina
- pain during sex
- pain during urination
- soreness or itching of the labia and inner thighs
- swollen labia

3.8.2 Pathophysiology

T. vaginalis principally infects the squamous epithelium of the genital tract. Incubation time is generally between 4 and 28 days. Infection may persist for long periods in females, but generally persists less than 10 days in males. Infection with *T. vaginalis* is a marker of high- risk sexual behavior. Co- infection with other STDs is common. In one study of adolescents who had a diagnosis of at least one STD, there was almost a 9-fold increase in the likelihood of *T. vaginalis* infection in the ensuing 3 months.

3.8.3 Trichomoniasis during pregnancy

T. vaginalis infection during pregnancy has been linked to preterm labor and low birth weight. Unfortunately, treatment of asymptomatic trichomoniasis has not been shown to prevent these outcomes. The decision to treat trichomoniasis during pregnancy is further complicated by the fact that physicians are reluctant to use metronidazole in pregnant women. As previously noted, however, the risk of teratogenicity appears to be overstated.

Risks and benefits of treatment must be considered in formulating a treatment plan for pregnant patients who have *T. vaginalis* infection. The CDC advises treatment of symptomatic pregnant women with a single 2 gm oral dose of metronidazole but does not recommend treatment of asymptomatic pregnant women (Owen and Clenney, 2004).

3.8.4 Complications

- Trichomoniasis is associated with increased risk of transmission of HIV.
- Trichomoniasis may cause a woman to deliver a low-birth-weight or premature infant. Additional research is needed to fully explore these relationships.
- In rare case, trichomoniasis in pregnant women may cause a premature rupture of the membranes and early delivery.
- Trichomoniasis is associated with an increased risk of inflammation of the fallopian tubes.

3.9 Other possible causes of vaginitis

3.9.1 Vaginal disease associated with increase associated levels of lactobacilli

A minority of clinicians believes that an overgrowth of the normal vaginal lactobactobacilli may cause symptoms of irritation, itching and discharge. These bacteria are the primary cause of the normally acid pH of the vagina. Furthermore, these two possible conditions are both termed vaginitis because of the lack of white blood cells on vaginal wet mounts, but each is characterized by inflammatory symptoms of itching or irritation (Libby, 2004).

3.9.2 Cytolytic vaginosis (Doderlein cytolysis)

Cytolytic vaginosis represents an abnormal increase in lactobacilli (Cibley *and* Cibley, 1991). Other clinicians believe this to result from hyperproliferation of vaginal epithelium of unknown cause. Patients with Cytolytic vaginosis describe itching, irritation, and a thick, clumped white vaginal discharge similar that seen with typical vaginal candidiasis. However, there is no redness or other signs of inflammation. Because of the abundance of lactobacilli, the pH is 4.5 or lower (Libby, 2004).

The diagnosis is confirmed on a microscopic examination of wet mount of vaginal secretions, but phase microscopy may be required. Vaginal secretions exhibit abundant lactobacilli and large number of epithelial cells, many with nuclei missing surrounding cytoplasm. These nuclei are similar in size to white blood cells, so that phase microscopy may require for differentiation. White blood cells, are not increased in number. Cytolytic vaginosis is nearly identical to candidiasis symptomatically and clinically. Lactobacilli vaginosis also produces itching, irritation, and a similar discharge. Alkalinization of the vagina relieves the symptoms of cytolytic vaginosis. A twice or thrice weekly douche using Sodium bicarbonate, 30-60 gm of baking soda in 1 liter of warm water can improve symptoms. This therapy is not curative and must be continued once or twice a week to control symptoms (Libby, 2004).

3.9.3 *Lactobacillus* vaginosis

An increase in numbers of lactobacilli to produce this recently described controversial condition (Horowitz *et al*, 1984). *Lactobacillus* vaginosis tends to occur in a setting of recent anticandidal therapy. Whether the therapy for yeast is causative, or whether empiric anticandidal therapy is inevitable as a first line treatment for women with these symptoms is not clear. Women with *Lactobacillus* vaginosis report itching and irritation, which they generally assume to be caused by yeast. In addition, they often describe a yeast like, white, thick, cheesy discharge is often present, and the pH of these vaginal secretions is normally acid .The diagnosis of this condition is based upon the symptoms of itching and irritation in combination with characteristic microscopic findings of vaginal secretions. These reveal very elongated lactobacilli, formally called “leptothrix”. There is no increase in white blood cells microscopically and there are no yeast forms. The diseases most often confused with *Lactobacillus* vaginosis are vaginal candidiasis and Cytolytic vaginosis. However, the microscopic appearance of the elongated lactobacilli is distinctive, and yeast forms are absent, as in cytolysis (Libby, 2004).

3.9.4 Atrophic vaginitis

Clinically significant atrophic vaginitis is uncommon; the majority of women with mild to moderate atrophy are asymptomatic. With reduced endogenous estrogen, the epithelium

becomes thin and lacking in glycogen and this contributes to a reduction in lactic acid production and an increase in vaginal pH. This change in the environment encourages the overgrowth of non-acidophilic coliforms and the disappearance of *Lactobacillus* spp. In women with advanced atrophy, symptoms include vaginal soreness, postcoital burning, dyspareunia, and occasional spotting. The vaginal mucosa is thin, with diffuse redness, occcasional petechiae, or ecchymoses with few or no vaginal folds. Vulvar atrophy may also be apparent, and the discharge may be serosanguineous or watery, with a pH of 5.0 to 7.0. The wet smear shows increased polymorphonuclear leukocytes associated with small, rounded parabasal epithelial cells. The *Lactobacillus* dominated flora is replaced by mixed flora of gram-negative rods, although bacteriologic cultures are unnecessary. Treatment consists of topical vaginal estrogen (Sobel, 1995).

3.9.5 Desquamative inflammatory vaginitis

Desquamative inflammatory vaginitis is an uncommon cause of an intractable vaginitis often accompanied by serious dyspareunia, which can occur at any stage of reproductive life and after the menopause. The cytological changes are identical with those seen in atrophic vaginitis yet the disorder often occurs in the presence of apparently normal ovarian function. Vaginal synechiae and stenosis developed in an appreciable number of patients. Treatment is unsatisfactory though there is some response to either local or systemic steroid therapy (Oates and Rowen, 1990).

3.9.6 Chemical vaginitis

Vaginitis may result from direct or allergic reactions to chemicals in spermicide used in barrier contraceptives or from agents contained in douches, deodorants, both preparations and certain condoms. Foreign bodies commonly cause vaginal discharge and infection in preadolescent girls. Paper, cotton, or other materials may be placed in the vagina and cause secondary infection. The clinical symptoms associated with foreign bodies include an abnormal vaginal discharge and intermenstrual spotting. Symptoms are generally secondary to dying of the vaginal mucosa and micro ulcerations, which can be detected by colposcopy. The treatment involves the removal of foreign body.

3.9.7 Vaginitis due to foreign body

A retained pessary or a forgotten tampon or swab can produce an offensive purulent discharge. Foreign bodies, which may be inserted deliberately, or accumulation of fluff or toilet tissue in the vagina of small children can cause vaginitis.

3.9.8 Vaginitis caused by excretions

Contamination of the vagina by urine (vesicovaginal fistula) or faeces (rectovaginal fistula) may produce a secondary vulvovaginitis. Poor hygiene may result in repeated *E. coli* infections. Similarly, serosanguineous discharge may arise from carcinoma in the vagina or cervix or from lesions higher in the genital tract and produce a secondary vaginitis.

3.10 Global scenario of BV, candidiasis and trichomoniasis

A study done by Marchetti *et al* in 1979 reported the prevalence of trichomoniasis to be 20.5%. In a study conducted among women attending a sexually transmitted disease (STD) clinic in Nairobi with vaginal discharge, *T. vaginalis, C. albicans* and *G. vaginalis* were found to be 34%, 24% and 75% respectively (Mirza *et al*, 1983). Similarly a study conducted among 247 women complaining of vaginal discharge in German found *G. vaginalis* in 62.8%, *Candida* spp. in 22.7% and *T. vaginalis* in 6.5% (Hoyme *et al*, 1984).

A study conducted by Diaz *et al* (1985) revealed that 21.8% had vaginitis caused by *Gardnerella*, 7.7% had candidiasis, and 2.8% had trichomoniasis; 1.1% were associated with *Gardnerella* and *Candida* and 0.55% had polymicrobial infection (both *Candida* and *Trichomonas*); and 66.1% patients did not show any kind of bacteria. Similarly, a study conducted in Sweden among 101 women found BV in 34 women (Holst *et al*, 1987). Likewise, in the study of Medical College in India, the most common cause of vaginal infection was BV by 29% followed by *Candida* by 18.8% and *T. vaginalis* by 11.32% (Archarya, 1988) and in a community study conducted by Bang *et al* (1989) from India found that 14% of women had trichomoniasis, 62% had BV and 34% had candidiasis.

Among 361 women with and 229 women without complaints of vaginal discharge in Denmark, *Candida* was found in 31.3% and 19.2%, *G. vaginalis* in 51.8% and 40.6% and *T*

vaginalis in 2.8% and 0.4% respectively. The BV was found in 35.7% women with and in 8.3% women without vaginal discharge (Bro, 1989). The total prevalence of BV was found to be 20.5% in Italy among seven hundred and ninety three women aged between 16 and 78 years (Cristiano *et al*, 1989). Similarly, a study in Washington conducted by Hillier *et al* (1993) among 171 pregnant women in labor at term found BV in 23% of the pregnant women.

The study from Egypt in 1993 showed that 18% of women had trichomoniasis, 22% had BV and 11% had candidiasis. Another study conducted by Ven-Dei Veen (1994) in Pikine (Senegal) among 147 women showed 82.9% had vaginal discharge.

Among 413 commercial sex workers in Calcutta, clinical diagnosis was found as candidiasis in 16.15%, trichomoniasis in 15.13% (Das, 1994). Similarly in a study among women with excessive vaginal discharge Mirza *et al* (1983) observed that the organism causing vaginitis were *G. vaginalis* in 75%, *T. vaginalis* in 34% and *C. albicans* in 24%. A study conducted by Harms (1995) in Madagascar among 231 women, 90% of women had vaginal discharge; the most prevalence was BV by 37% followed by trichomoniasis by 31% and candidiasis by 30%. Similarly, Meda (1995) has found vaginitis rate of 45%.

A study among infertile Nigerian women, candidiasis was found in 29.5% and trichomoniasis in 44.4% in secondary subfertile group whereas candidiasis in 12.5% and trichomoniasis in 4.0% in primary subfertile group were (Okonofua, 1995). In a study among 206 pregnant women attending Port Moresby (Papua New Guinea) General Hospital in 1990-1991, candidiasis was observed in 23% of women, *T. vaginalis* in 19% and BV in 23% (Klifio *et al*, 1995). Similarly, another study conducted in London by Evans (1995) found the prevalence of BV in 8.4%, vaginal candidiasis in 32.9% and trichomoniasis in 2.9%.

A study conducted among 168 asymptomatic pregnant women in Durban, South Africa revealed that 71% of women had vaginitis and 52% had BV (Govender *et al*, 1996). Similarly, another study in South Africa showed the vaginal infection in 20-49% of

antenatal and family planning clinic attenders. Among them, 60.9% showed vaginal discharge, 46.8% had BV and in 50% cases mixed infection of *Candida* and *Trichomonas* was found (Kanter, 1996).

A study conducted in Nairobi found vaginitis among 20% pregnant women. The study found the prevalences of Candidiasis, trichomoniasis and BV 26.2%, 19.9% and 20.6 % respectively (Thomas, 1996). Similarly, a study carried out among asymptomatic pregnant women at last trimester found BV in 4.9% pregnant women (Cristiano *et al*, 1996). The study conducted in Jakarta among 4519 pregnant women found 18% had BV and 3.8% had trichomoniasis (Joesoef *et al*, 1996). A study conducted in Jamaica among 269 pregnant women found that 18% had trichomoniasis, 44.1% had BV and 30.7% had candidiasis (Kamara *et al*, 2000).

In the study conducted by Kurnatowska and Mamos from 1955-1999, the prevalence of *T. vaginalis* was observed from 26.6% to 78.0% and on the last ten years it was found in about half of the sample examined women (Kurnatowska and Mamos, 2001). Similarly, a study conducted in Nigeria among women with vaginal discharge found *T. vaginalis* in 74.5% women (Anorlu *et al*, 2001). Another study among married Jordanian women complaining of vaginal discharge found that 29.7% of patients had BV (Abu-Shaqra, 2001). A study conducted by Verghese *et al* (2001) Chennai, India found *C. albicans* infection rate 40.47%. Mathew *et al* (2001) in the same place found 38.5% BV in symptomatic antenatal women. Candidiasis was the most frequent infection in 1998, detected in 22.5% of the tests (Adad *et al*, 2001).

A constant decreasement of *T. vaginalis* positive specimens from 19% to fewer than 2 % was observed in a hospital in Denmark during 1967 to 1997 (Dragsted *et al*, 2001). A study in Venezuela found that 28.5% had vulvovaginitis; *Candida* spp. was present in 84.2% and *T.vaginalis* in 14% (Azzam *et al*, 2002). Similarly a study conducted in Nigeria found the prevalences of *Candida* spp., *T. vaginalis* and *G. vaginalis* to be 37%, 4.7% and 3.9% respectively (Aboyeji and Nwabuisi, 2003).

A study conducted by Sirimai *et al* in Siriraj Hospital found the prevalence of BV to be 14.6%, in which asymptomatic disease was recognized in up to 47.9% (Sirimai *et al*, 2004). Shimano *et al* (2004) conducted a study in Japan to evaluate the prevalence of BV in 2004 and showed 18.2% infection rate over the 8 years period. The annual rate of BV increased from 13.6% in 1993 to 21.4% in 2000 and in Zabre (2004) Kazmierczak *et al* conducted a study and found candidiasis in 42%, BV in 19% and *T. vaginalis* in 4%. Pickering *et al* in southeast Uganda in 2005 found BV (36.1%), *T. vaginalis* (18.9%) and candidiasis (18.6%).

Namkinga *et al* in Tanzania (2005) observed candidiasis in 45%, BV in 48.4% and *T. vaginalis* in 93%. Similarly, Gutman *et al* (2005) in USA found the prevalence of BV in 38.7%. A study conducted in Quetta among 500 women found vaginitis in 33.48%, BV in 30.7%, candidiasis in 10% and trichomoniasis in 7.2% women (Sami and Baloch, 2005). A study of vaginal discharge specimens from women aged 15-50 years in Saudi Arabia found the prevalence rate of trichomoniasis to be 0.7% (Alzanbagi *et al*, 2005).

In Tamil Nadu, India, the prevalence of trichomoniasis, BV and vaginal candidiasis has been found to be 13%, 18% and 10% respectively (Helen *et al*, 2005). Similarly in the study conducted among 1223 pregnant women from Amir-Almomenin General Hospital in Semnan, Iran, the prevalence for BV and *T. vaginalis* were detected in 16.0% and 5.5% of these women respectively (Azargoon and Darvishzadesh, 2006).

In United States, a study conducted from 1992 to 1995 among a cohort of 212 women with HIV in Los Angeles County, trichomoniasis was found in 17.4% of women and reported as the most frequently identified sexually transmitted disease (Sorvillo *et al*, 1998). Similarly, a study conducted among HIV infected and HIV uninfected women found BV, *T. vaginalis* and yeast vaginitis in 42.8%, 6.1% and 10.0% HIV-infected women whereas the rate was 47.0%, 7.8% and 3.8% in HIV uninfected women (Watts *et al*, 2006). Similarly, Mbizvo *et al* (2004) in Zimbabwe found the prevalence of BV 36% among HIV seropositive women and 26% among seronegative.

In Denmark, a study was conducted among 2,927 pregnant women and BV was diagnosed in 13.7% of Danish pregnant women (Thorsen *et al,* 2006). Similarly, a study was conducted among 493 patients in Spain between December 1998 and February 2000. The prevalence of *Candida* spp. was found to be of 28%. Among the *Candida* spp, *C. albicans* was the most prevalent and found to be of 90.4% (Garcia *et al,* 2006).

A study conducted among 500 antenatal attendees (gestational age < or = 20 weeks) attending two hospitals serving urban areas in Vientiane, between September 2001 and March 2002, *Candida* spp. had the highest prevalence of all infections having of 27.0%, followed by BV of 14.4% by Amsel's criteria and 22.0% by Nugent's score and *T. vaginalis* of 1.8% (Sihavong *et al,* 2006).

In a study conducted by Patel *et al* in 2006 in Goa, India BV in 17.8% and candidiasis in 8.5% were found. Similarly, a study conducted in Uganda found *T.vaginalis* in 8% girls (Rassjo *et al,* 2006). Another study in Peru, Patricia *et al* found BV in 35.7%, *T. vaginalis* in 5.9% and *C. albicans* in 7.7%. Similarly the mixed infection rates for BV and *T. vaginalis* was 39.0% and BV, *T.vaginalis* and *C. albicans* was 44.9% (Patricia *et al*, 2006). In Peru, Garcia *et al* (2006) has found the mixed infection rate of 39% for BV and *T. vaginalis* and 7.7% candidiasis among symptomatic women.

In a study conducted in Nepal, the prevalence of BV was found to be 69% in symptomatic women and 62% in asymptomatic women. It also showed the prevalence of trichomoniasis to be 14.1% (Aryal, 1997). Similarly, in a study conducted in 500 women of reproductive age from January 2004 to July 2004 at gynaecological out patient department of Tribhuvan University Teaching Hospital, BV was found in 2.5%. Among all BV positive patients, 67.3% were symptomatic and 32.7% were asymptomatic (Manandhar *et al*, 2006). Similarly, Rizvi and Luby (2004) in Nepal found BV in 25% and trichomoniasis in 17%.

CHAPTER-IV

4 MATERIALS AND METHODS

4.1 Materials

A list of materials used in this study is given in Appendix-II.

4.2 Study design

A cross-sectional descriptive study was done to determine the prevalence of BV, vaginal candidiasis and Trichomoniasis among the pregnant women visiting Maternity Thapathali Hospital, Nepal.

4.3 Study site

The study site was selected the clinical pathology Laboratory of "Thapathali Maternity hospital", Thapathali, Kathmandu.

4.4 Study period

The study was conducted during the period of 14th Jestha, 2063 to 18th Aswin, 2063.

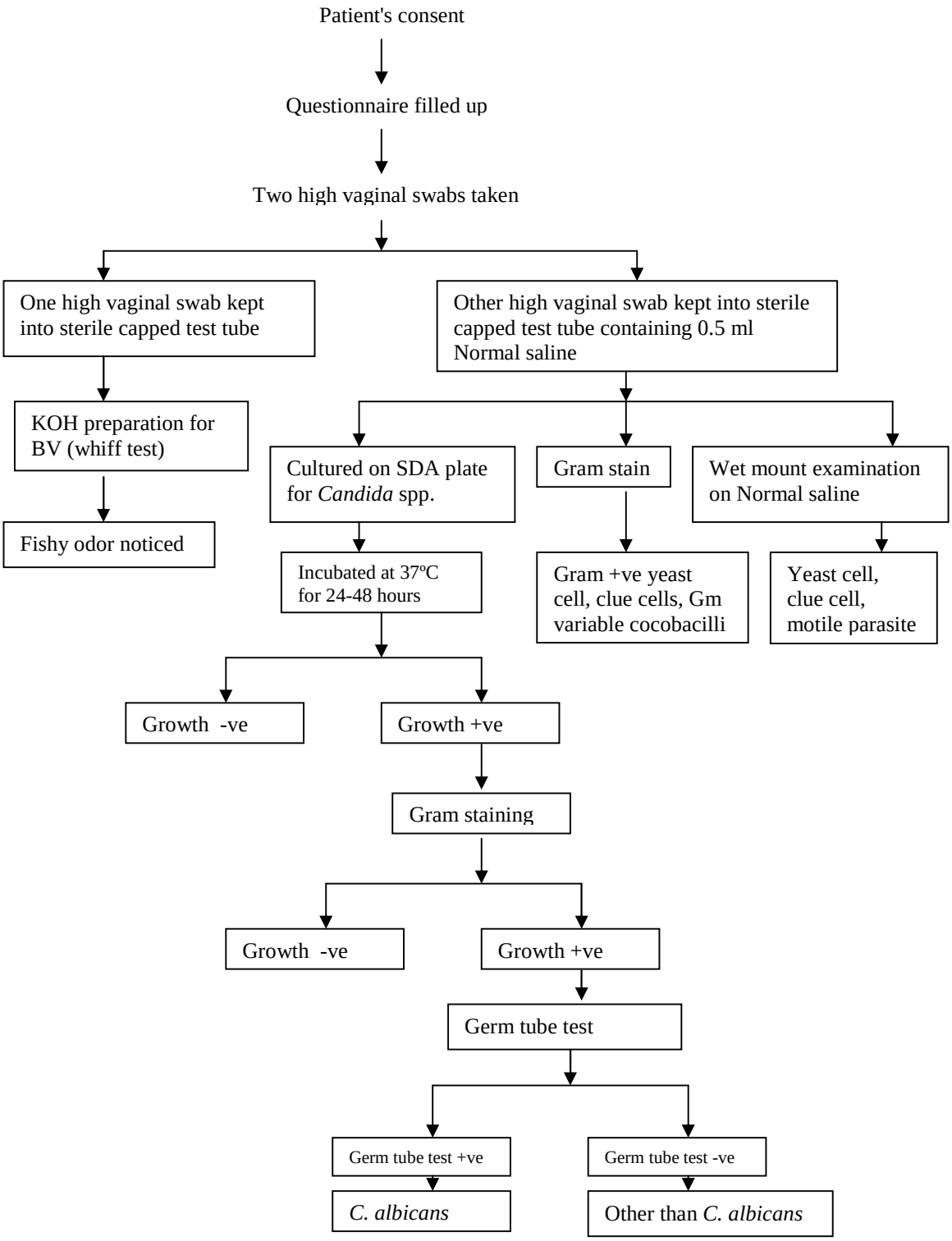
4.5 Target population

200 women of reproductive age group who were pregnant and visiting Thapathali Maternity hospital were enrolled for the study.

4.6 Data collection and analysis

Primary data were collected from the population for both qualitative and quantitative purposes. Opened self prepared questionnaire was administered to collect the data from pregnant women. The collected data was then analyzed for statistical purposes by using appropriate statistical tools.

Flow chart of the procedure



4.7 Consent

Oral/ verbal consent was taken from all patients included in the study before proceeding the Questionnaire and specimen collection.

4.8 Laboratory diagnostic procedure

4.8.1 Specimen collection

Gynecologist, trained nurse and metron helped in collecting two vaginal swabs from the posterior fornix of the pregnant women. Two high vaginal swabs were collected from the pregnant women visiting Thapathali Maternity hospital. The sterile cotton swabs were provided from laboratory.

4.8.1.1 Transportation of specimen

One high vaginal 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.

4.8.2 Sample processing

Each sample was examined as follows

4.8.2.1 Microscopy

Of two collected HVS, one swab was used for the preparation of whiff test and another swab was used for culture, wet mount and gram stain.

4.8.2.1.1 Whiff test

i. The HVS was rolled on the clean and grease free new slide.
ii. A drop of 10% KOH was added to the slide.
iii. The slide was hold close to the nose to detect the amine odour.

iv. The specimen would quickly become odorless upon standing so the odor should be noted as soon as possible.

If fishy smell noticed, the sample was considered as positive.

4.8.2.1.2 Wet mount microscopy

It was indicated for *T. vaginalis,* clue cells and yeast cells.

Procedure for wet mount microscopy

Following steps were performed orderly

- New and non greasy slide and cover slip was taken
- Drop of normal saline was added to the center of the slide
- Thin and homogenous suspension was made
- The suspension was covered with cover slip
- Checked by low power and than by high power objective

Making the preparation too thick was avoided.

Examination must be completed before drying of the preparation.

4.8.2.1.3 Gram's stain microscopy

It was indicated for clue cells and yeast cells.

Procedure for Gram staining

The Procedure for Gram's staining is given in Appendix-IV

4.8.2.1.4 Culture

The culture was only done for confirming *C. albicans.*

i. The Petri plate having SDA media was taken and labeled.
ii. The HVS was than taken and the primary inoculum was made at one corner without touching the border of the plate.
iii. With the sterile loop the streaking was done starting from the primary inoculum.
iv. Then the plate was incubated in incubator at 37 ºC for 24 – 48 hours.

v. Cream-colored pasty colonies would appear if *C. albican* was present. So the plate was observed after 24 hours to 48 hours.

vi. The colonies were gram stained and if the yeast cells were seen then it was proceed for the germ tube test.

4.8.2.1.5 Germ tube test (Cheesbrough, 2000)

- 500 micro liter of human serum was pipetted into small test tube.
- Using a sterile wire loop, the serum with a yeast colony from the SDA plate was inoculated. The tube was placed in water bath or incubator at 35 - 37º C for 2-3 hours.
- Using the pasture pipette, a drop of serum yeast culture was transferred to a glass slide, and covered with a cover glass.
- The preparation was examined using the 10X and 40X objectives with the condenser iris diaphragm closed sufficiently to give good contrast.
- Sprouting yeast cells, that is tube-like outgrowths from the cells, known as germ tubes were examined

If sprouting yeast cells were seen, the culture was reported as 'C. *albicans* isolated'.

4.8.3 Safe disposal of sample and contaminated tools

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

CHAPTER V

5 RESULTS

In this study, 200 high vaginal swab samples were collected from the pregnant women visiting Thapathali Maternity Hospital from 14th Jestha to 14th Aswin and the samples were processed in emergency laboratory of the hospital. Among 200 pregnant women visiting Thapathali Maternity Hospital, 78 (39%) had vaginitis (Figure 4).

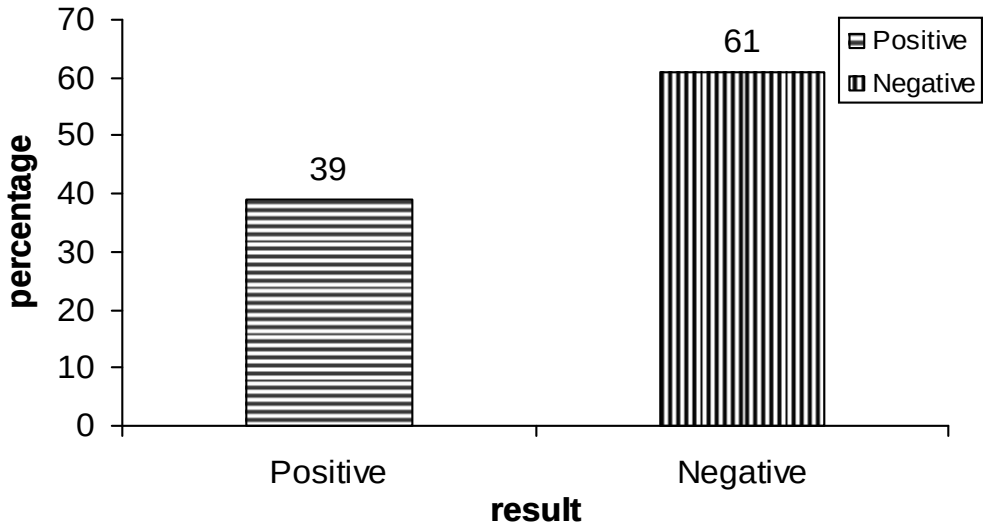


Figure 4 Prevalence of vaginitis among the pregnant women visiting Thapathali Maternity Hospital

Table 2 Types of infection detected among the pregnant women

Infection types	**Frequency**	**Percent**
C. albicans infection	23	29.48
Bacterial vaginosis	41	52.56
C. albicans + Bacterial vaginosis	12	15.38
C. albicans + Bacterial vaginosis + *T. vaginalis*	1	1.28
T. vaginalis infection	1	1.28
Total	78	100

Of the positive result, 29.48% had candidiasis, 52.56% had BV and 1.28% had trichomoniasis (Table 2), showing BV most prevalent.

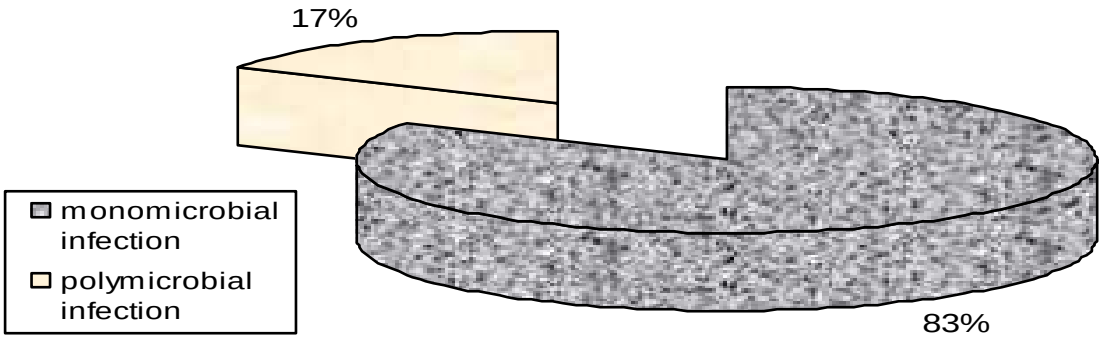


Figure 5 Pattern of vaginal infection among pregnant women

Of 78 positive samples, 83.3% had monomicrobial infection and 16.7% had polymicrobial infection (Figure 5).

Table 3 Distribution of vaginitis in pregnant women of different ethnic groups

Ethnic groups	Total	Positive	Percentage	P-value
Indo-Aryan	97	39	40.2	P>0.05
Tibeto-Burman	103	39	37.8	
Total	200	78	39	

Out of 200 samples, 97 (48.5%) were Indo-Aryans and 103 (51.5%) were Tibeto-Burmans. Among Indo-Aryans, 39 (40.2%) were found to be infected where as among Tibeto-Burmans only 39 (37.8%) were found to be infected (Table 3). However, the difference was statistically insignificant.

Table 4 Distribution of vaginitis in pregnant women of different settlement area

Area	Total	Positive	Percentage	P-value
Rural	42	19	45.2	
Urban	158	59	37.3	P>0.05
Total	200	78	39	

Among total study population, 42 (21%) were from rural area and 158 (79%) were from urban area. The positive infection rate for rural area was 45.2% and for urban area was 37.3% (Table 4). Though the rates were high in rural area than urban area, the difference was not statistically significant (P>0.05).

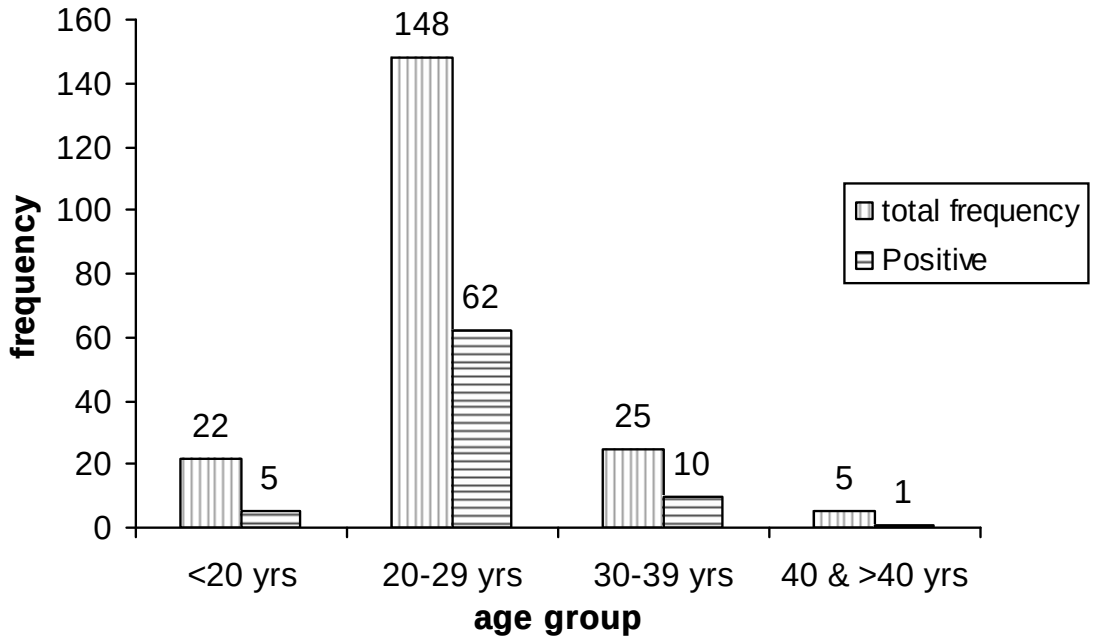


Figure 6 Age wise distribution of vaginitis among pregnant

In the study, among 22 women of less than 20 years, 5 (22.72%) were infected, out of 148 pregnant women included in age group 20 to 29 years, 62 (41.89%) were infected, among 25 women of age group 30-39 years, 10 (40.0%) were infected and of 5 pregnant women in the age group 40 and more than 40 only 1(20.0%) were infected.

Table 5 Age wise distribution of organism in infected women

Age group	Total	Organism					Positive total	Positive %
		C. albicans	BV	*C. albicans* + BV	*C. albicans* +BV+ *T. vaginalis*	*T. vaginalis*		
<20 yrs	22	1	3	1	0	0	5	22.72
20-29 yrs	148	17	31	11	1	1	56	41.89
30-39 yrs	25	5	5	0	0	0	12	40.00
40&>40 yrs	5	0	1	0	0	0	2	20.00
Total	200	23	41	12	1	1	78	39.00

The age wise distribution of vaginitis showed that the women of age group 20 to 29 years were mostly infected and least infected age group was 40 and more than 40 years. BV being the most prevalent in all groups followed by *C. albicans* and other as shown in Table 5. This difference was not statistically significant (P>0.05).

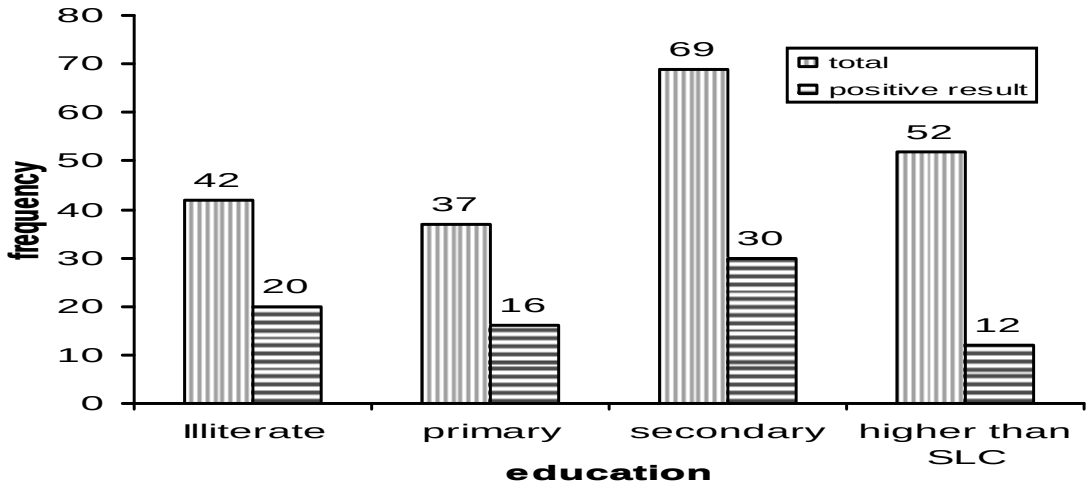


Figure 7 Occurrence of vaginitis according to their educational status

In the study, among 42 illiterate pregnant women, 37 women having primary education, 69 women having secondary education and 52 women having education higher than SLC, 20, 16, 30 and 12 had vaginal infection respectively (figure 7).

Table 6 Pattern of vaginal infection in relation to educational status

Education	Total	Organism					Positive total	Positive %
		C. albicans	BV	*C. albicans* + BV	*C. albicans* +BV+ *T. vaginalis*	*T. vaginalis*		
Illiterate	42	5	9	2	0	0	20	47.6
Primary	37	7	8	3	1	1	16	43.2
Secondary	69	8	17	5	0	0	30	43.5
Higher than SLC	52	3	7	2	0	0	12	23.0
Total	200	23	41	12	1	1	78	39

The infection rate was highest among illiterate pregnant women (47.6%) and least among the women having education higher than SLC (23.0%). However, the result was statistically insignificant (P>0.05)**.** Prevalence of organisms according to their educational status in pregnant women was found as in Table 6.

Table 7 Distribution of organisms in vaginitis in relation to the occupation

Occupation	Total	Organism					Positive total	Positive %
		C. albicans	BV	*C. albicans* + BV	*C. albicans* + BV + *T.vaginalis*	*T. vaginalis*		
Agriculture	43	9	10	1	1	0	21	48.8
Housewife	132	13	23	9	0	1	46	34.8
Other than agriculture	25	1	8	2	0	0	11	44.0
Total	200	23	41	12	1	1	78	39.0

In the study, highest numbers of patients (132) were housewives and least number of patients (25) had occupation other than agriculture where as 43 women were agriculture

based. Distribution of organisms in vaginitis in relation to the occupation was observed as in Table 8. The study showed 48.8% women with the agriculture as their occupation, 34.8% housewives and 44% women with the occupation other than agriculture had vaginal infection.

Figure 8 Prevalence of vaginitis in pregnant women in relation to their occupation

The rate of infection was highest for the pregnant women who were agriculture based and least for the housewives (Figure 8).

Table 8 Occurrence of vaginal infection type in relation to the occupation

Occupation	Infection type		Total
	monomicrobial infection	polymicrobial infection	
Agriculture	19	2	21
Housewife	37	9	46
Other than agriculture	9	2	11
Total	65	13	78

Among housewives, 37 had monomicrobial infection and 9 had polymicrobial infection. Likewise, among agriculture based women, 19 had monomicrobial infection and 2 had

polymicrobial infection. Among the women whose occupation was other than agriculture, 9 had monomicrobial infection and 2 had polymicrobial infection (Table 7). This difference was also statistically insignificant (P>0.05).

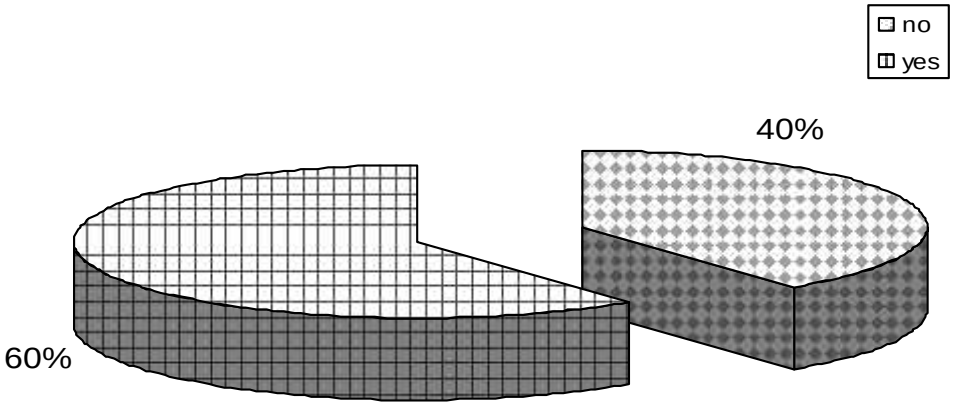


Figure 9 Percentage of pregnant women having vaginal discharge

Out of 200 pregnant women, 121 (60.5%) women had vaginal discharge and 79 (39.5%) women didn't have such discharge (Figure 9).

Table 9 Occurrence of infection with vaginal discharge

Vaginal discharge	Total	Result		Positive percent	P-value
		Negative	Positive		
Absent	79	67	12	15.2	P<0.05
Present	121	55	66	54.5	
Total	200	122	78	39	

Among women having discharge, 54.5% had vaginal infection whereas only 15.2% of pregnant women having no discharge were infected. The difference was statistically significant (P<0.05).

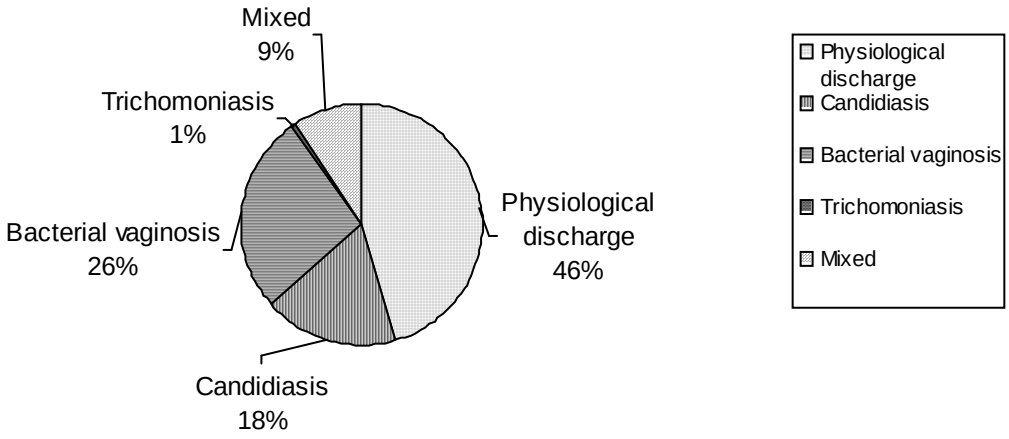


Figure 10 Occurrence of infection in pregnant women with vaginal discharge

Among 121 pregnant women having vaginal discharge, 66 (54.54%) had infection that is pathological discharge and 55 (45.45%) had physiological discharge. Among women having physiological discharge, 18.18%, 26.44%, 0.82% and 9.09% had candidiasis, BV, trichomoniasis and mixed infection respectively (Figure 10).

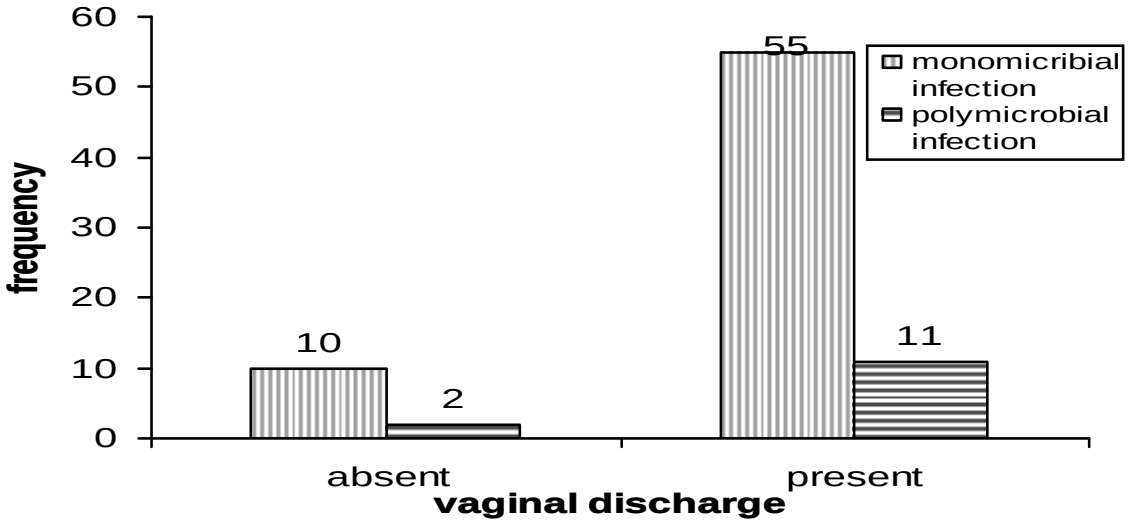


Figure 11 Occurrence of infection types in relation of discharge

The vaginal infection was also present in 12 women who did not have any discharge; 10 women had monomicrobial infected and 2 had polymicrobial infection. The vaginal infection was more in those having discharge. Among 66 women having discharge, 55 women had monomicrobial infection and 11 had polymicrobial infection (Figure11).

Table 10 Duration of vaginal discharge and infection type

Duration of discharge	Total	Negative	Infection type		Positive total	Positive %
			Monomicrobial infection	Polymicrobial infection		
no discharge	79	67	10	2	12	15.2
less than 2 weeks	98	40	45	13	58	59.2
2weeks to 1 month	3	2	1	0	1	33.3
1 month to 6 month	8	4	4	0	4	50.0
6 month to 1 year	7	4	3	0	3	42.8
1 year to 3 year	3	3	0	0	0	0.0
more than 3 year	2	2	0	0	0	0.0
Total	200	122	65	13	78	39.0

Out of 121women having discharge, the highest number of women 98 had vaginal discharge for less than 2 weeks. The rate of infection was also highest in the pregnant women having discharge for less than 2 weeks (Table 10).

Table 11 Distribution of organism in relation to the type of vaginal discharge

Types of discharge	Organisms					Total
	C. albicans	BV	*C. albicans* + BV	*C. albicans* + BV+ *T. vaginalis*	*T. vaginalis*	
No discharge	1	9	2	0	0	12
White curdy	21	2	5	0	0	28
Mucopurulent	0	10	1	0	1	12
Serous watery	1	18	0	0	0	19
Blood mixed	0	0	1	0	0	1
mixed	0	2	3	1	0	6
Total	23	41	12	1	1	78

Among 78 pregnant women having positive result, 28 had white curdy, 12 had mucopurulent, 19 had serous watery discharge and other as shown in Table 11 and it also shows BV most prevalent.

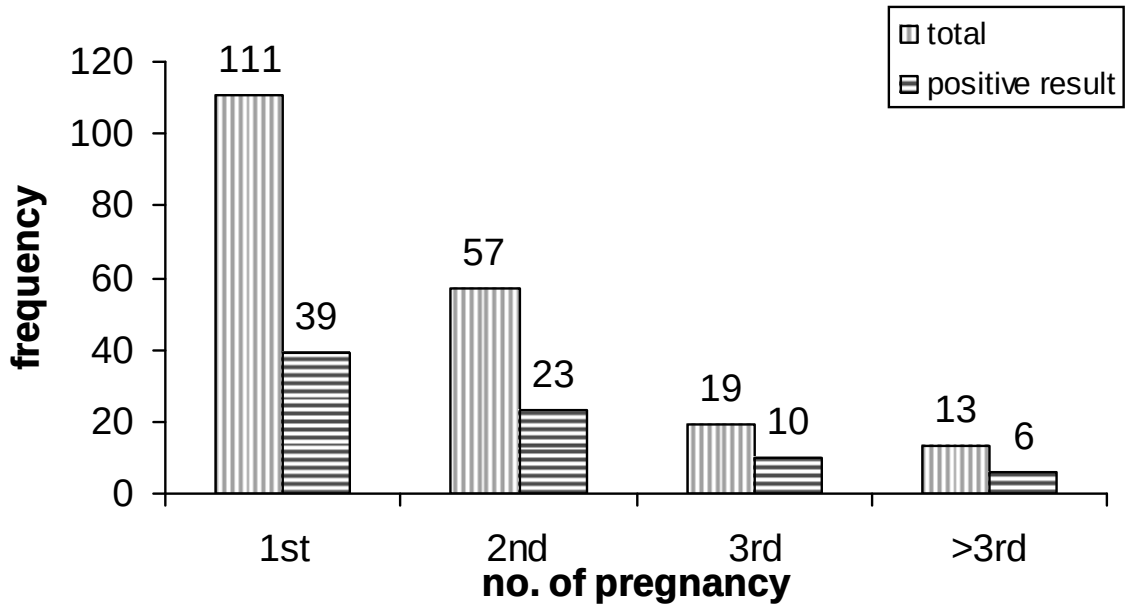


Figure 12 Distribution of vaginal infection in relation to the number of pregnancy

Out of 200 patients, 111 (55.5%) had 1st pregnancy, 57 (28.5%) had 2nd, 19 (9.5%) had 3rd and 13 (6.5%) had more than 3rd pregnancy. Among them 39, 23, 10 and 6 were infected (Figure12). Among total, the rate of infection was highest in the women having 3rd number of pregnancy (52.6%) and lowest for the women having 1st number of pregnancy (35.1%).

Table 12 Occurrence of organism with number of pregnancy

Number of pregnancy	Total	Organism					Positive	Positive %
		C. albicans	BV	*C. albicans* + BV	*C. albicans* + BV + *T.vaginalis*	*T. vaginalis*		
1st	111	9	21	8	0	1	39	35.1
2nd	57	8	10	4	1	0	23	40.3
3rd	19	4	6	0	0	0	10	52.6
>3rd	13	2	4	0	0	0	6	46.1
Total	200	23	41	12	1	1	78	39.0

Of the positive result, the occurrence of organisms with number of pregnancy is shown in table 12 showing bacterial vaginosis most prevalent.

Table 13 Occurrence of vaginitis in relation to sign and symptoms

Sign and symptoms	Result		Total	Percent of infected	P-value
	Negative	Positive			
Asymptomatic	101	53	154	34.41	P<0.05
Symptomatic	21	25	46	54.34	
Total	122	78	200	39	

Of 200 total samples, 154 (77.0%) were asymptomatic and 46 (23%) were symptomatic which showed symptoms like irritation, itching and inflammation. The positive result was found among 53 & 25 and the rate of infection was 34.41% & 54.34% among asymptomatic

and symptomatic pregnant women respectively showing the rate of infection was highest for the symptomatic women. The difference was statistically significant (P< 0.05) (Table 13).

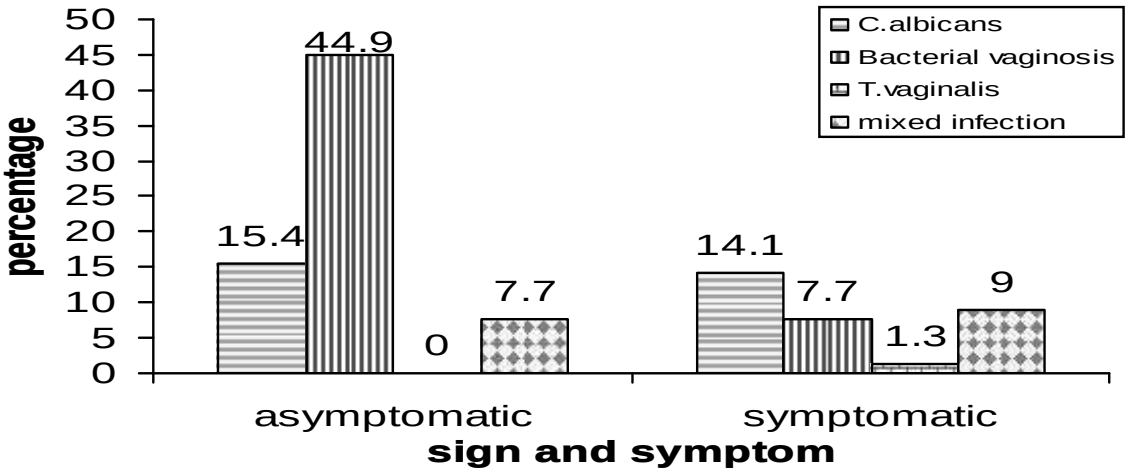


Figure 13 Distribution of organisms in relation of sign and symptom

Among the asymptomatic, BV was the most prevalent (44.9%) followed by candidiasis (15.4%) and mixed infection (7.7%) but no trichomoniasis. Similarly, among the symptomatic pregnant women candidiasis was the prevalent (14.1%) followed by BV and candidiasis + BV both 7.7% and than candidiasis + BV + *T.vaginalis* and *T. vaginalis* both by 1.3% (Figure 13).

Table 14 Pattern of infection in relation to the symptoms

Types of infection	Types of patients				P-value
	Symptomatic N=46		Asymptomatic N=154		
	No.	%	No.	%	
Monomicrobial infection	18	39.13	47	30.5	P>0.05
Polymicrobial infection	7	15.2	6	3.89	

Among the pregnant women having the positive result, the monomicrobial infection was predominant than polymicrobial infection both in symptomatic and asymptomatic cases (Table 14).

CHAPTER-VI

6 DISCUSSION AND CONCLUSION

6.1 Discussion

Vaginitis literally refers to any infection or inflammation of the vagina, most often caused by a bacteria, fungi or parasites. The cause is usually a change in the normal balance of vaginal flora or due to any infection. Many things can disturb the balance of a healthy vagina, including douching, feminine hygiene sprays, certain soaps or bubble baths, antibiotics, diabetes, pregnancy or infections. In some cases, vaginitis results from organisms that are passed between sexual partners. Vaginal symptoms are one of the most common reasons for gynecological consultation. The most common types of vaginitis are BV, vaginal candidiasis, and trichomoniasis (Owen and Clenney, 2004).

Abnormal vaginal discharge, changes in quantity, color and odor compared with normal vaginal discharge may indicate vaginitis. It has been reported that one in three women in Nepal wants consultation for vaginal discharge (Pradhan, 2003). Vaginitis causes different types of morbidity in women especially of reproductive age group (Aryal, 1997). Vaginitis may result in sterility, abortion, and stillbirth. Since the disease leads to many complications, it is of the prime importance to know the causative organisms, prevalence of the disease and different factors that can affect the prevalence among Nepalese women. Women are more prone to infection during pregnancy. In Nepal, very few studies have been implicated in the prevalence and therapeutic consideration of vaginitis in pregnant women. Therefore, this cross-sectional descriptive study was planned to determine the prevalence of BV, vaginal candidiasis and trichomoniasis among the pregnant women visiting Thapathali Maternity Hospital.

The study was conducted during the period of 14th Jestha, 2063 to 14th Aswin, 2063.Two hundred pregnant women visiting Thapathali Maternity Hospital were included for the study. This study was carried out in the clinical pathology laboratory of Thapathali Maternity hospital because the flow of pregnant women is higher in this Maternity hospital.

The pregnant women of all reproductive age groups were considered as the target group of our study and the study was restricted to the pregnant women visiting the hospital. Oral/verbal consent was taken from all patients enrolled in the study before administration of the questionnaire and specimen collection. A self-designed questionnaire was prepared and short interview was taken with those pregnant women regarding their health, number of pregnancies, socioeconomic status, education, types of discharge, duration of discharge, sign and symptoms etc. Then the vaginal swabs were taken and were taken to microbiology laboratory of clinical pathology laboratory of Thapathali Maternity Hospital for processing. The data were then analyzed by using appropriate statistical tools.

It has been found from the present study that among 200 pregnant women 78 (39%) were found to have infectious vaginitis. Sami and Baloch (2005) in Ouetta found similar type of result (33.5%). Thomas (1996) stated lower percentage (20%) of vaginitis in Nairobi where as very high percentage (71%) in South Africa. Among total positive cases in the study, BV was the most common (52.56%) followed by candidiasis (29.48%) and trichomoniasis was detected in 1.3% of the positive sample. Two or more of these infections co-existed in 16.7%**.** In the study, the prevalence of BV was highest followed by candidiasis and then by trichomoniasis.

The trend of infection of this study was similar to the study conducted by Bang *et al* (1989) in India, Bro (1989) in Danmark, Acharya (1988) in India, Kamara *et al* (2000) in Jamaica, Sami and Baloch (2005) in Ouetta and Patricia *et al* (2006) in Peru but was in contrary to the study conducted by Evans (1995) in London, Thomas (1996) in Nairobi. In their studies, the highest prevalence was for candidiasis followed by BV and trichomoniasis. The highest prevalence of candidiasis in their studies was reported to be due to the intake of oral contraceptive, IUCD, previous history of candidiasis, or other. Leegard (1984) found a significant association between wearing an IUCD and candidiasis.

Pickering *et al* (2005) and Helen *et al* (2005) found the highest prevalence in BV followed by trichomoniasis and candidiasis in Southeast Uganda and Tamilnadu respectively. Namkinga *et al* (2005) in Tanzania found the highest prevalent of trichomoniasis followed

by BV and then by candidiasis. Eschenbach *et al* (1988) reported that most of the cases of infectious vaginitis were caused by nonspecific vaginitis, which accounted for 40-50%; candidiasis, which accounted for 20-30%; and trichomoniasis, which accounted for 20-30%. Two or more of these infections may co-exist so that the presenting sings and symptoms may not always be typical. This change in the trend might be due to the differences in the living standards, socio-cultural status, hygienic condition, awareness towards their health, education and so on.

BV was previously called nonspecific vaginitis (Gillespie, 1994). BV is currently, the most prevalent cause of infectious vaginitis among women attending for genitourinary diseases (Gonzalez *et al*, 1999). Research suggests that BV is actually not an infection but as a condition resulting from an imbalance in the vaginal flora (Pradhan, 2001). BV accounts for 10 to 30 percent of cases of infectious vaginitis in women of childbearing age (Owen and Clenney, 2004). All pregnant women who have ever had a premature delivery or low birth weight baby should be considered for a BV examination, regardless of symptoms, and should be treated if they have BV. All pregnant women who have symptoms of BV should be checked and treated.

The prevalence of BV in the present study was 52.56% which is very much consistent to the study conducted by Govender *et al* (1996) in South Africa. The finding was higher than the study conducted by Gutman *et al* (2005) in USA (38.7%), Garcia *et al* (2006) in Peru (39%), Mathew *et al* (2001) in Chennai (38.5%) and lesser than the study conducted by Bang *et al* (1989) in community study of India (62%). This might be due to the difference in their living condition, education, smoking habit, health awareness etc.

In this study the infection rate of BV was found in 44.9% and 7.7% among asymptomatic and symptomatic cases of pregnant women respectively. BV is commonly found in asymptomatic women (Owen and Clenney, 2004). Aryal (1997) in Nepal also found BV as most common infection in asymptomatic which was similar to this study but not for the symptomatic cases. Among symptomatic, most common was candidiasis (14.1%). Kanter (1996) found 46.8% in STD clinic attenders, 55.5% in family planning clinic attenders and

60.9% in symptomatic women with vaginal discharge attending gynae OPD in different places study of South Africa. Mathew *et al* (2001) in Chennai found 38.5% BV among symptomatic which was higher than the prevalence among similar group in this study. Aryal (1997) in Nepal showed 69% and 62% BV, Manandhar *et al* (2005) in Kathmandu 67.3% and 32.7% among symptomatic and asymptomatic women respectively while Cristiano *et al* (1989) found 4.9% BV among asymptomatic pregnant women which was lesser than this study.

After BV, candidiasis was found to be the causative agent of vaginitis. During pregnancy, the vagina shows increased susceptibility to infection by *Candida*, resulting in both a higher prevalence of vaginal colonization and a higher rate of symptomatic vaginitis (White and Larsen 1997). High levels of reproductive harmones provide an excellent carbon source for *Candida* spp. by providing higher glycogen content in the vaginal tissues. A more complex mechanism is likely, in that estrogen enhances adherence of yeast cells to the vaginal mucosa. A cytosol receptor or binding system for female reproductive hormones has been documented in *C. albicans. S*everal investigators demonstrated in vitro binding of female sex hormones to *Candida* spp. as well as the capicity of certain hormones to enhance yeast mycelia formation and hence virulence (Zhao *et al*, 1995).

The prevelance of candidiasis in this study was 29.4% which was nearly similar to study conducted by Evans (1995) in London (32.9%), Kamara *et al* (2000) in Turkey (27%), Sihavong *et al* (2006) in Vientiane (27.0%) and Bajracharya (2004) in Nepal (26.86%). The prevelence of candidiasis was higher in Nigeria, Venezula, Tanzania and Chennai shown by Aboveji and Nwabuiri (2003), Azzam *et al* (2002), Namkinga *et al* (2005) and Verghese *et al* (2001) respectively but the findings observed by Basnet (2004) in Nepal (16.12%), Mirza *et al* (1983) in Nairobi (24%), Hoyme *et al* (1984) in German (22.7%), Diaz *et al* (1985) in colombia (7.7%), Acharya (1988) in India (18.8%), Das (1994) in India (16.15%) and Mirza *et al* (1995) in Kenya (24%) were lower than the study. This might be due to the difference in their habit of using oral contraceptives, IUCD and other reasons.

Unlike the previous two types of vaginitis, trichomoniasis is primarily a sexually transmitted disease in which the disease is passed from person-to-person primarily by sexual contact. Its prevalence is very low in present study subjects (1.28%). This might be due to the pregnant women included in this study were not of high risk group. Other reasons could be that during the examination, they might have already taken antibacterial or antifungal drugs which might have shown antimicrobial effect. However, further study is suggested for finding specific and accurate reasons. Almost similar result were shown in the study conducted by Alzanbagi *et al* (2005) in Saudi Arabi (0.7%) and Sihavong *et al* (2006) in Vientiane (1.8%) but lesser than the findings obtained by Evans (1995) in London (2.9%), Thomas (1996) in Nairobi (19.9%), Joesoef *et al* (1996) in Jakarta (18%), Aboveji and Nwabuisi (2003) in Nigeria (4.7%), Azzam *et al* (2002) in Venezula (14%), Kamara *et al* (2000) in Turkey (8.1%) and Azargoon and Darvishzadeh (2006) in Iran (5.5%). Nimkinga *et al* (2005) in Tanzania showed extremely higher rate of trichomoniasis (93%) than the study. Infection with *T. vaginalis* is frequently associated with other sexually transmitted diseases and helps spread the HIV virus (Sorvillo *et al*, 2001).

This study revealed higher infection rate of vaginitis among the pregnant women of Indo-Aryan (45.2%) ethnicity as compared to Tibeto-Burman (37.8%); however the difference was not statistically significant. This indicated that ethnic aggregation and predisposition to the vaginal infection is not genetically determined but may be strongly determined by the behavioural factors, socioeconomic condition, health awareness etc. The finding was consistent with the finding of Kevin and Mark (2004) and Manandhar *et al* (2005) among the in-patient of reproductive age group attending gynecological out patient department of T.U Teaching hospital.

In this study, a higher rate of vaginal infection was found among the pregnant women from the rural area (45.2%) than from urban area (37.3%). Similar finding was obtained by Manandhar *et al* (2005). Although the result was found statistically insignificant, differences may be caused by differences in sexual norms and practices which may affect exposure to infection as well as by differences in willingness to report symptoms or be

examined. Women in rural area may be more secluded and live in a more conservative sexual milieu by contrast social values in urban areas allow women more sexual freedom.

It has also been found from the present study that the occurrence of the vaginal infection was found to be more among the women having agriculture as their occupation (48.8%) followed by women with the occupation other than agriculture (44%) and housewife (34.8%). However, this was not statistically significant. The higher infection rate among farmers can be correlated with the higher infection rate among the rural females as most of the farmers represent the rural areas. So, the poor sanitation practices, lack of time to keep their proper health, poor living standard, ignorance and difficulty in accessibility towards immediate health care facilities may attribute to the higher rate of vaginitis among farmers from the rural region.

The study also revealed higher prevalence of vaginitis among the illiterate pregnant women (47.6%) and least for the pregnant women who had education above SLC had (23.0%). Similar result were found by Manandhar *et al* (2005) and Patricia *et al* (2004).The low economic status, lack of education, lack of a female doctor at the health service centers and the socio culture structure might be regarded as the cause of higher prevalence of vaginitis among less-educated women and housewives. Prasad (2005) in India reported that culture of silence shrouds gynecologic morbidity throughout India and elsewhere.

In this study, 200 high vaginal swabs were obtained from the pregnant women who were from the age range 18 to 50 years. Among them, 11.0%, 74%, 12.5% and 2.5% were from age group less than 20 years, 20 to 29 years, 30 to 39 years and 40 and above 40 years respectively. The infection rate was highest among pregnant women of age group 20 to 29 years (71.79%) and least for 40 and above 40 years group (2.6%). Similarly, Bajracharya (2004) found vulvovaginal candidiasis most prevalent among 20 to 25 years group (41.17%) and least in 35 to 40 years group (13.04%).

The highest prevalence in the age group 20 to 29 might be due to the age being the most reproductively active age group and the high sexual exposure at this age. It is extremely rare

before menarche, and its annual incidence increases dramatically towards the end of the 2nd decades of life and peaks over the next two decades. Young women are particularly susceptible to infection because they have fewer antibodies to fight against pathogens (Prasad, 2005). The incidence of vaginitis decreases after 40 years. This might be because during this period, vaginal dryness occurs or it may be because they have less sexual activity. The finding obtained by Helen *et al* (2005) was somewhat different than this study in which the highest prevalent of RTIs among 16 to 22 years was reported. Similarly, Manandhar *et al* (2005) observed the highest prevalence of vaginitis among the age group 25 to 29 and least below 20 years age group which was consistent to the above study.

It has also been found that 121 (60.5%) of the pregnant women had vaginal discharge and 79 (39.5%) did not have such discharge. Among the women having discharge, 54.5% had vaginal infection whereas only 15.2% having no discharge was infected. This showed that vaginal discharge is also one of the diagnostic criteria for the vaginitis as the difference was statistically significant in the study.

In the study, pathological discharge was found in 66 (54.54%) and physiological discharge in 55 (45.45%) out of 121 pregnant women presented with vaginal discharge which was different than in the study of Narjis and Stephen (2004). They found pathological discharge in 91% out of 70 women who presented vaginal discharge. Although the physiological discharge is common in pregnant women, our study revealed pathological discharge in 54.54% of pregnant women, which may be due to lower education, low socioeconomic status and personnel unhygienic practices.

From the study, it is clear that the chance of getting vaginitis increases with the frequency of pregnancy. Here, the rate of infection is in the order 3rd> 2nd>1st pregnancy. This might be due to the vaginal stasis increases with number of pregnancy and sexual exposure. Other reason might be with increase in number of pregnancy, the physical health of the women becomes weaker leading to the physical disabilities, reduced immunity power and decreases their hygiene and sanitation interest.

In this study, it was found that 154 (77.0%) were asymptomatic and 46 (23%) were symptomatic but the rate of infection was more among the symptomatic cases of pregnant women (54.34%) as compared to asymptomatic cases (34.41%). However, the difference was not found statistically significant. It also showed that BV was the most prevalent among asymptomatic cases of pregnant women which corelates to the statement given by Owen and Clenney, (2004) whereas candidiasis among symptomatic. The pattern of infection in relation to the symptoms showed monomicrobial infection was common in both cases of symptomatic and asymptomatic cases.

Although the study is novel in our country and provides important information, there are some important limitations that affect discussion.

- Due to time factor and other constraints, the study had to be confined over the limited sample size. More significant findings would have been resulted if more gynae OPD, STDs clinic and other health services of different places were included.
- Due to lack of resources, the culture was done only for *C. albicans* not for the different organisms of BV and *T. vaginalis*. If their culture were done more significant findings would have resulted.
- The findings cannot be extrapolated to the entire pregnant population of Nepal due to the confounding variables as the study was only base in the pregnant women who visited Thapathali Maternity hospital of Nepal.
- This study had included only the pregnant women therefore the non-pregnant women should also be included so that the prevalence of common types of vaginitis among women could be studied.
- The antibiotic susceptibility couldn't be studied due to the provision of the specific antibiotic disc in the laboratory; if it would have been studied the correct antibiotic could have been revealed for proper treatment of vaginitis.
- The different causative organisms like *G. vaginalis* and other anaerobes were not identified separately due to lack of anaerobic culture media and other resources.

6.2 Conclusions

Hence, the common type of vaginitis was predominant among the sexually active group of patients. The age, clinical sign and symptoms, vaginal discharge, number of pregnancy occupation and education were found to pose a remarkable risk to contribute the vaginitis. Vaginitis was predominant among the pregnant women having vaginal discharge and other sign and symptoms. The pathological discharge was higher in pregnant women than physiological discharge. The monomicrobial infection was also most common than polymicrobial infection in the infected pregnant women. Bacterial vaginosis was the most prevalent followed by candidiasis and trichomoniasis among pregnant women visiting “Thapathali Maternity hospital”, Nepal.

CHAPTER-VII

7 SUMMARY AND RECOMMENDATIONS

7.1 Summary

- The vaginal swabs were collected from 200 pregnant women visiting Thapathali Maternity Hospital, among them 78 (39%) had vaginitis. Of the positive result, 29.48% had candidiasis, 52.56% had BV and 1.28% had trichomoniasis, showing BV most prevalent and 83.3% had monomicrobial infection and 16.7% had polymicrobial infection.

- Among Indo-Aryans, 40.2% were found to be infected whereas, among Tibeto-Burman, only 37.8% were found to be infected. The positive infection rate for the women from rural area was 45.2% and urban area was 37.3%.

- Pregnant women included of age group 20 to 29 years in this study were mostly infected (41.8%) and least infected group was of 40 and more than 40 years (20.0%). The infection rate was highest among illiterate women (47.6) and least among the women having education higher than SLC (23.0%).

- The rate of infection was highest for the pregnant women who were involved in agriculture as occupation (48.8%) and least for the housewife (34.8%). Among women having discharge, 54.5% had vaginal infection whereas; only 15.2% of pregnant women having no discharge were infected.

- The pathological discharge was found in 54.54% and physiological discharge in 45.45% in pregnant women presented with vaginal discharge. The rate of infection was highest in the women having 3rd pregnancy (52.6%) and lowest for more than 1st number of pregnancy (35.1%).

- The positive result of vaginitis was found among 53 & 25 and the rate of infection was 34.41% & 54.34% among asymptomatic and symptomatic cases of pregnant women respectively. Among the asymptomatic cases, BV was the most prevalent (44.9%) and among the symptomatic cases candidiasis was the most prevalent (14.1%).

7.2 Recommendations

- It is recommended to conduct study on pregnant women including large population, possibly at national level based on different antenatal clinic, OPD wards, family planning services centers and postnatal check up clinics etc. to find out the prevalence of common types of vaginitis among women.

- The study should be continued for the occurrence of vaginitis in relation to the antibiotic users, OD and IUCD users so that the clear pattern of corelation of these factors with vaginitis could be resulted.

- Several sexual and hygienic practices including clothing and bathing have been implied in pathogenesis, therefore the study on sexual habits and hygienic conditions should be studied.

- Antibiotic susceptibilities pattern should be studied to suggest susceptible antibiotics so that pregnant women with vaginitis must be treated with proper antibiotics that are safe for mother and fetus.

- Basic health knowledge and awareness programme must be lunched in national level to women in order to minimize the occurrence of vaginitis.

- The study of the correlation of vaginitis with the stages of pregnancy is recommended for the improvement of women's health and for prosperity of the nation.

- Many other advanced techniques like PCR, DNA probe methods must be recommended for more specificity and sensitivity.

- In our context, most common cause for vaginal discharge in pregnant women was pathological not physiological so the study strongly recommends for medical attention.

CHAPTER-VIII

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APPENDIX-I

QUESTIONNAIRE

Form no. _____ Code no._____ Date _____

General

1 Identification

1.1 Name of patient

1.2 Address

1.3 Age

1.4 Education a. illiterate b. Primary
c. Secondary d. Higher than SLC

1.5 Occupation a. agriculture b. housewife
c. other than agriculture

Specific

2 Obstetric history

2.1 No. of pregnancy

2.2 Cijnical sign and symptoms

a. irritation b. itching
c. inflammation d. more than one symptom

2.3 Previous history

i. Have you ever had sores/ ulcer around your private parts?

a. yes b. no c. don't know

ii. Has your husband ever had sores/ ulcer around his private parts?

a. yes b. no c. don't know

iii. Have you ever had any discharge?

a. yes b. no c. don't know

iv. Has your husband ever had any discharge?

a. yes b. no c. don't know

v. Presenting excessive vaginal discharge

a. mucopurulent b. white curdy c. serous watery d. blood mixed

vi. Duration of discharge

a. less than 2 wks b. 2 weeks-1 month c. 1 month-6 month

d. 6month-1 year e. 1 year- 3 years f. > 3 years

vii. Smell of discharge

a. normal b. offensive c. fishy odour

If 2.3 i, ii, iii and iv are answered 'yes' then process with question 2.4. If answered as 'no' and 'don't know' then stop to question 2.4.

2.4 i. Did you have treatment?

a. yes b. no

ii. Where did you get treatment?

a. hospital/ health post b. clinic c. pharmacy d. other

iii. Did you have treatment?

a. yes b. no

iv. Where did you get treatment?

a. hospital/ health post b. clinic c. pharmacy d. other

If 2.4 i and iii are answered 'yes' then question 2.5 are asked

i.What sort of treatment did you get?

a. injection b. tablet c. capsule d. other

ii.What sort of treatment did your husband get?

a. injection b. tablet c. capsule d. other

APPENDIX-II

DIFFERENT MATERIALS USED DURING THE RESEARCH PERIOD WERE LISTED BELOW

Glasswares

i. Petri plates
ii. Conical flasks
iii. Measuring cylinder
iv. Test-tubes
v. Beakers
vi. Slides
vii. Coverslipes
viii. Glass rod

Media (Hi Media)

i. Sabourad’s Dextrose Agar

Reagents

i. Crystal violet
ii. Iodine
iii. Methanol
iv. Safranin
v. 10%Potassium hydroxide
vi. Normal saline (0.9% NaCl)
vii. Lysol

Equipments

i.	Incubator	Heraeus D-6450, Hanau.
ii.	Refrigerator	Godrej, Cold-gold.
iii.	Autoclave	ELCON
iv.	Water bath	Napco, 220 model.
v.	Microscope	Leitz. Biomed.
vi.	Centrifuge	Kubota model KC 25, Japan.
vii.	Hot-air oven	Ambassadors, Laboratory Electronic Oven.
viii.	Gas burner	

Miscellaneous

i. Loop
ii. Candle
iii. Cotton-tipped swabs
iv. Heater
v. Magnetic stirrer
vi. Gloves
vii. Cotton

APPENDIX-III

COMPOSITION AND METHOD OF PREPARATION OF DIFFERENT CULTURE MEDIA AND REAGENT USED

I CULTURE MEDIA

Different types of culture media such as selective media and transport media were used. Composition and preparation of different types of culture media are given below. All the media used are supplied by Hi media company and final pH of all media at 25ºC is 7.4 ± 0.2.

1 Sabouraud Dextrose Agar

Ingredients	**gm/litre**
Mycological peptone	10.0
Dextrose	40.0
Agar	15.0
Final pH (at 25ºC)	5.6± 0.2

65.0 grams of power medium was suspended in 1000 ml distilled water and than boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121ºC (15 lbs pressure) for 15 minutes.

II REAGENT

1. GRAM'S STAINING REAGENT

a. Crystal violet (Hucker's modification)

Solution A	Crystal violet	20g
	Ethyl alcohol	200ml
Solution B	Ammonium chloride	8g
	Distilled water	800ml

Solution A and B were mixed and filtered.

b. Iodine solution(Gram Iodine)

Iodine crystal	1g
Potassium iodide	2g
Distilled water	300ml

Iodine crystal and potassium iodide were grinded in a mortar. A few ml of water was at a time and grinded thoroughly after each addition until the solution was achieved. The solution was rinsed with the remainder of the distilled water, and kept into an amber glass bottle.

c. Decoloriser

Acetone	100ml
Ethyl alcohol	500ml

Note: Other acetone-alcohol ratio may be used (e.g 1:4, 1:2, 1:1). Higher the acetone content the more rapidly bacteria will be decolorized.

d. Safranin (counter stain)

Stock solution	Safranin O	10g
	Ethyl alcohol	200ml
Working solution	Stock solution	100ml
	Distilled water	900ml

2. Potassium hydroxide

10% potassium hydroxide was prepared by dissolving 5 gram of KOH crystals in 50 ml of sterile distilled water.

3. Normal saline

0.9% normal saline was prepared by dissolving 0.9 gram of Sodium chloride crystals in 100 ml of sterile distilled water.

APPENDIX-IV

STANDARD METHODOLOGY OF GRAM'S STAINING

The Gram's staining technique is as follows

- The smear fixed slide was taken.
- The smear was covered with crystal violet stain for 30-60 seconds.
- The stain was rapidly washed with clean water. (If tap water is not clean, the filtered water or clean boiled rain water.)
- All water was tipped off, and the smear was covered with Lugol's iodine for 30-60 seconds.
- Iodine was washed off with clean water.
- Decolorized rapidly (few seconds) with acetone-alcohol. Washed immediately with clean water.
- The smear was covered with neutral red stain for 2 minutes.
- The stain was washed off with clean water.
- The back of the slide was wiped clean, and placed in draining rack for the smear to air-dry.
- The smear was than examined microscopically, first with 40X objective to check the staining and to see the distribution of material, and than with the oil immersion objective to look for bacteria and cells.

(Cheesbrough Monica pp 31)

APPENDIX-V

Features of the most common causes of vaginitis

Basis of diagnosis	Bacterial vaginosis	Vulvovaginal candidiasis	Trichomoniasis
Signs and symptoms	Thin, off-white discharge, unpleasant ”fishy” odor, with odor increasing after sexual intercourse	Thick, white(“cottage cheese”) discharge with no odor	Pruritus vaginal irritation, dysuria, no symptoms in 20 to 50 percent of affected women
Physical examination	Usually normal, appearance of tissue, discolored discharge with abnormal odor, homogeneous discharge that adheres to vaginal walls	Vulvar and vaginal erythema, edema and fissures, thick, white discharge that adheres to vaginal walls	Vulvar and vaginal edema and erythema, “strawberry” cervix in up to 25% of affected women , forthy, purulent discharge
Laboratory tests			
Vaginal pH (normal <4.5)	Elevated(>4.5)	Normal	Elevated(>4.5)
Microscopic examination of wet mount and vaginal discharge	“Clue cells”(vaginal epithelial cells coated with coccobacilli, few lactobacilli, occasional motile, curved rods (*Mobiluncus species*)	Pseudohyphae, mycelial tangles or budding yeast cells	Motile trichomonads, many polymorphonuclear cells

“Whiff” test (normal=no odor)	Positive	Negative	Can be positive
Additional tests	Amsel’s criteria (three of four criteria must be met); provides correct diagnosis in 90% of affected women. Criteria of Nugent or Spiegel for Gram to diagnose the bacterial vaginosis other tests are controversial	KOH Microscopy Gram stain Culture	DNA probe tests: sensitivity of 90 percent and specificity of 99.8 percent Culture: sensitivity of 98 percent and specificity of 100 percent

KOH=potassium hydroxide

Information derived from Carr PL, Friedman RH. Evaluation and management of vaginitis. J Gen Intern, 1998; 13:335-46, and Sobel JD. Vaginitis. N Eng. J. med 1997: 337: 1896-903

APPENDIX-VI

Causes of Vaginitis

Type of vaginitis	**Causes and comments**
Valvovaginal candidiasis	*C. albicans, C. glabrata, C. tropicalis*
Bacterial vaginosis	*Gardnerella vaginalis*, *Mycoplasma hominis*, *Mobiluncus* spp, *Bacteriods* spp (other than *Bacteriods fragilis*)
Trichomaniasis	*Trichomanas vaginalis*
Atrophic vaginitis	Estrogen deficiency
Chemical irritation	Soaps, hygiene products(tampons, sanitary napkins, latex condoms)
Lichen planus (desquamative type)	flat, hyperkeratotic lesions that are pruritic or painful; associated vulvar and oral lesions
Allergic vaginitis	Sperm, douching, hygiene products(tampons, sanitary napkins, latex condoms or diaphragms), dyes, inhaled allergens, occupational exposures
Foreing body with or without infection or trauma	Tampons, contraceptive device, pessary, other

(Marion and Timothy, 2004)

APPENDIX-VII

Classification of Vaginitis

Variable	**Uncomplicated disease***	**Complicated disease****
Symptom severity	Mild /moderate	Severe
Frequency	Sporadic/infrequent	Recurrent
Organisms	Candida albicans	Species other than *Candida albicans*
Host factors		
Immune	Normal	Abnormal (e.g. uncontrolled diabetes mellitus or immunosupression)
Pregnancy status	Non –pregnant	pregnant

*Patients must have all these factors

** Patients may have any one of these factors

(Eschenbach D. A, 2004)

APPENDIX-VIII

Differential Diagnosis of Vaginitis

Variables	Normal	Vulvovaginal Candidiasis	Bacterial Vaginosis	Trichomoniasis
Symptoms	None or mild, transient	Pruritus, soreness, change in discharge, dyspareunia	Malodourous discharge, no dyspareunia	Malodourous purulent discharge, dyspareunia
Signs		Vulvar erythema, edema, fissure	Adherent discharge	Purulent discharge, vulvovaginal erythema
pH	4.0-4.5	4.0-4.5	>4.5	5-6.0
Amine test	Negative	Negative	Positive (~70-80%)	Often positive
10% KOH examination	Negative	Pseudohyphae (~70%)	Negative	Negative
Miscellaneous	-	Culture if microscopy negative	Culture of no value	Culture if microscopy negative
Differential diagnosis	Physiological leucorrhea	Contact irritant or allergic vulvitis, chemical irritation, focal vulvitis (vulvodynia)		Purulent vaginitis, Desquamative inflammatory vaginitis, atrophic vaginitis plus secondary infection

(Sobel, 1997)

APPENDIX-IX

PREVENTION GUIDELINES FOR VAGINAL INFECTIONS

- Have new partners wear condoms during sexual intercourse.
- Stay healthy; eat well, get enough sleep, drink enough fluids.
- Keep vaginal area clean and dry.
- Wear cotton underwear.
- Wipe from front to back after urination or bowel movement.
- Avoid using deodorant pads or tampons.
- Don't use petroleum jelly or other oils for lubricants.
- Don't douche.
- Use medication as long as directed.
- Avoid sexual intercourse until treatment is completed and you are symptom free.
- Don't scratch infected or inflamed areas; it can cause further irritation.
- If using medication inside the vagina, use it during the menstrual period.
- During an infection, use pads rather than tampons if menstruation occurs.
- Avoid vulvo/vaginal irritants, including perfumed or deodorant soaps/body washes.
- If symptoms persist after completing the treatment, an exam is indicated. Call for an appointment, and please use nothing in the vagina for 48 hours prior to your exam.

APPENDIX-X

DATA ANALYSIS (CHI-SQUARE TEST)

A. Association of vaginal infections and different ethnic groups of pregnant women

Infection / Ethnic group	Presence of vaginal infections	Absence of vaginal infections	Total
Indo-Aryan	39	58	97
Tibeto-barman	39	64	103
Total	**78**	**122**	**200**

Test statistic is χ^2

Ho: There is no significant association of vaginal infections and different ethnic groups

H_1: There is significant association of vaginal infections and different ethnic groups

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=0.115$

Thus χ^2 cal (0.115) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 1 i.e.3.841

Hence, Ho is accepted i.e. there is no significant association of vaginal infections and different ethnic groups of pregnant women [P>0.05].

B. Association of vaginal infections and different settlement areas of pregnant women

Infection / Settlement area	Presence of vaginal infections	Absence of vaginal infections	Total
Rural	19	23	42
Urban	59	89	158
Total	**78**	**122**	**200**

Test statistic is χ^2

Ho: There is no significant association of vaginal infections and different settlement areas

H_1: There is significant association of vaginal infections and different settlement areas

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=0.861$

Thus χ^2 cal (0.861) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 1 i.e.3.841

Hence, Ho is accepted i.e. there is no significant association of presence of vaginal infections in women of different settlement areas [P>0.05].

C. Association of vaginal infections and different age groups of pregnant women

Infection / Age group	Presence of vaginal infections	Absence of vaginal infections	Total
<20 years	8	14	22
20-29 years	56	92	148
30-39 years	12	13	25
40 and >40years	2	3	5
Total	**78**	**122**	**200**

Test statistic is χ^2

Ho: There is no significant association of vaginal infections and different age groups

H_1: There is significant association of vaginal infections and different age groups

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=0.98$

Thus χ^2 cal (0.98) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 3 i.e.7.815

Hence, Ho is accepted i.e. there is no significant association of vaginal infections and different age groups of pregnant women [P>0.05].

D. Association of vaginal infections and different educational status of pregnant women

Infection / Education	Presence of vaginal infections	Absence of vaginal infections	Total
Illiterate	16	21	37
Primary	20	22	42
Secondary	30	39	69
Higher than SLC	12	40	52
Total	**78**	**122**	**200**

Test statistic is χ^2

Ho: There is no significant association of vaginal infections and educational status

H_1: There is significant association of vaginal infections and educational status

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=7.69$

Thus χ^2 cal (7.69) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 3 i.e.7.815

Hence, Ho is accepted i.e. there is no significant association of vaginal infection and educational status of pregnant women [P>0.05].

E. Association of types of vaginal infections and different occupation of pregnant women

Infection type / Occupation	Presence of monomicrobial infection	Presence of polymicrobial infection	Total
Agriculture	19	2	21
Housewife	37	9	46
Other than agriculture	9	2	11
Total	**65**	**13**	**78**

Test statistic is χ^2

Ho: There is no significant association of vaginal infection and different occupation

H_1: There is significant association of vaginal infection and different occupation

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=1.28$

Thus χ^2 cal (1.28) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 2 i.e.5.991

Hence, Ho is accepted i.e. there is no significant association of vaginal infections with different educational status of pregnant women [P>0.05].

F. Association of vaginal infections and sign and symptoms in pregnant women

Infection / Sign & symptoms	Presence of vaginal infections	Absence of vaginal infections	Total
Asymptomatic	53	101	154
Symptomatic	25	21	46

Total	**78**	**122**	**200**

Test statistic is χ^2

Ho: There is no significant association of vaginal infections and vaginal discharge

H_1: There is significant association of presence of vaginal infections and vaginal discharge

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=30.60$

Thus χ^2 cal (30.60) > χ^2 tab at $\alpha=0.05$ and degree of freedom= 1 i.e.3.841

Hence, H_1 is accepted i.e. there is significant association of presence of vaginal infections and vaginal discharge in pregnant women [P<0.05].

G. Association of presence of vaginal infections and vaginal discharge in pregnant women

Infection / Vaginal discharge	**Presence of vaginal infections**	**Absence of vaginal infections**	**Total**
Present	66	55	120
Absent	12	67	80
Total	**78**	**122**	**200**

Test statistic is χ^2

Ho: There is no significant association of vaginal infections and vaginal discharge

H_1: There is significant association of vaginal infections and vaginal discharge

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=30.60$

Thus χ^2 cal (30.60) > χ^2 tab at $\alpha=0.05$ and degree of freedom= 1 i.e.3.841

Hence, H_1 is accepted i.e. there is significant association of vaginal infections and vaginal discharge in pregnant women [P<0.05].

H. Association of types of vaginal infections and sign and symptoms

Infection type / Occupation	**Monomicrobial infection**	**Polymicrobial infection**	**Total**
Asymptomatic	47	6	53

Symptomatic	18	7	25
Total	**65**	**13**	**78**

Test statistic is χ^2

Ho: There is no significant association of vaginal infection and different occupation

H_1: There is significant association of vaginal infection and different occupation

From $\chi^2=\Sigma (0-E)^2/E$ we find $\chi^2=3.44$

Thus χ^2 cal (3.44) < χ^2 tab at $\alpha=0.05$ and degree of freedom= 2 i.e.3.841

Hence, Ho is accepted i.e. there is no significant association of vaginal infections with different educational status of pregnant women [P>0.05].